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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES
AND TOXIC SUBSTANCES

MEMORANDUM

DATE: May 15, 2006

SUBJECT: **Etofenprox – DER Review** Neurotoxicity Studies

PC Code: 128965
DP Number: 297157
TXR Number 0052279

REVIEWER: Meta Bonner, Toxicologist Meta Bonner 5-15-06
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The following data evaluation records were completed for etofenprox: Developmental Neurotoxicity Study – Rats (MRID 46062301), Acute Oral Neurotoxicity Study – Rats (MRID 45932301), and Subchronic Neurotoxicity Study – Rats (MRID 45925701).

JUN 21 2006

DATA EVALUATION RECORD

ETOFENPROX (MTI-500)/ 128965
[OPPTS 870-6200a (§81-8)]

STUDY TYPE: ACUTE ORAL NEUROTOXICITY STUDY IN RATS
MRID 45932301

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
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Task Order No. 18-2004

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Template version 11/01

TXR: 0052279

DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity - Rats [OPPTS 870.6200a (§81-8)] OECD 424.

PC CODE:128965 **DP BARCODE:** D297157
SUBMISSION NO.: N/A

TEST MATERIAL (PURITY): MTI-500 (Etofenprox) (99%)

SYNONYMS: Etofenprox, Ethofenprox , RF 316 , SAN 811 I , Zoecon RF-316 , 2-(4-Ethoxyphenyl)-2-methylpropyl 3-phenoxybenzyl ether, Benzene, 1-((2-(4-ethoxyphenyl)-2-methylpropoxy)methyl)-3-phenoxy- (9CI) (CA INDEX NAME)

CITATION: Smith, Peter B. (2002) Acute oral gavage neurotoxicity study with MTI-500 in rats. Covance Laboratories, Inc. (Madison, WI). Laboratory report number, Covance 6648-154, 9 August 2002. MRID 45932301. Unpublished.

SPONSOR: Mitsui Chemicals, Inc. (Chiyoda-ku, Tokyo, Japan)

EXECUTIVE SUMMARY: In an acute neurotoxicity study (MRID 45932301), groups of fasted, 53-61 day old Crl:CD®(SD)IGS BR rats (10/sex) were given a single oral dose of MTI-500 (etofenprox, 99% a.i., lot# 87031) in 1.0% methylcellulose in reverse osmosis water at doses of 0, 25, 125, 500, or 2000 mg/kg bw and observed for 15 days. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed in 10 animals/sex/group prior to dosing and at Days 1, 8, and 15 following MTI-500 exposure. At study termination, all animals were euthanized and perfused *in situ* for neuropathological examination. Of the perfused animals, six animals/sex/group from the control and highest dose groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

There were no treatment related effects on mortality, clinical signs, body weight, brain weight or gross and histologic pathology or neuropathology. FOB and motor activity testing revealed no treatment related effects.

Based on the results of this acute oral neurotoxicity study, the NOAEL for MTI-500 (etofenprox) in male and female rats is 2000 mg/kg (limit dose). The LOAEL was not identified (>2000 mg/kg).

This neurotoxicity study is classified **Acceptable/Guideline** and **satisfies** the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424). Minor deficiencies: The FOB methods do not state whether the same observer was used throughout testing. Neither the environmental conditions nor the test equipment used were described in the FOB study report. FOB recordings were not made for some required parameters: arousal/general activity levels, convulsions, and tremors. The positive control data are from the year 1996 and should be updated.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided. Prestudy procedures, such as general husbandry and health screen activities, were conducted starting two days prior to protocol finalization. This was the only GLP deviation reported and did not affect either the outcome or the interpretation of the data.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: MTI-500

Description:	Off-white to light yellow solid; protect from light; store at room temperature
Lot/Batch #:	87031
Purity:	99 % a.i.
CAS # of TGAI:	80844-07-1
Structure:	Not given in the study report.

2. Vehicle and/or positive control: 1.0% methylcellulose in reverse osmosis (RO) water; the prepared vehicle was stored in a refrigerator set to maintain 2-8°C. The test article was purged with nitrogen gas and heated in an incubator set at 60°C until it was in liquid form. The required amounts of test article were weighed and added to a formulation container containing control vehicle. The suspension was transferred to a graduated cylinder and the formulation container was rinsed with control vehicle. The rinse was transferred to the cylinder and additional control vehicle was added to reach the final volume. The contents of the cylinder were transferred back to the formulation container and the suspension was placed in a water bath (40°C) and mixed using a magnetic stir plate and stir bar for approximately 15-20 minutes or until a visually uniform suspension was formed. The dose preparations were dispensed into amber glass containers for each dosing day. The container was purged with nitrogen gas and maintained in a water bath (40°C) overnight.

3. Test animals:

Species:	Rat	
Strain:	Crl:CD (SD) IGD BR	
Age/weight at dosing:	53-61 days old/ males: 208-347 g; females: 150-219 g	
Source:	Charles River Laboratories (Portage, MI)	
Housing:	Individually in suspended stainless steel cages	
Diet:	Certified rodent diet (#8728C, Harlan Teklad) *ad libitum*, except when fasted for dosing and necropsy	
Water:	*ad libitum*	
Environmental conditions:	Temperature:	19-25°C
	Humidity:	30-70%
	Air changes:	10/hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	22-24 days	

B. STUDY DESIGN:

1. **In life dates**: Start: 29-31 January 2002; End:13-18 February 2002

2. **Animal assignment and treatment**: Animals were assigned to the test groups noted in Table 1 using a computerized blocking procedure to achieve body weight balance with respect to treatment group. At the time of randomization, the weight variation was within 2.1 standard deviations of the mean body weight for each sex. Following an overnight fast, rats were given a single dose by oral gavage in 1% methylcellulose in reverse osmosis water, 10 mL/kg. Rats then were observed twice daily for mortality and moribundity and weighed weekly for 15 days (on Days 1, 8, and 15). Dose levels were chosen based on the upper limit required by regulatory guidelines (2000 mg/kg bw); other dose levels were set as sequential fractions of the high dose. Gavage was used because a potential route of exposure in humans is by the oral route. Estimated time to peak effect was approximately 5 hours after dosing. Administration was staggered over 3 days to facilitate neurobehavioral observations. Approximately equal numbers of animals/ treatment group were dosed on each day. Whether males and females were treated on separate days is not specified in the report. Survivors were sacrificed and necropsy was performed. Cholinesterase levels were not determined.

TABLE 1. Study design

Experimental parameter	Dose group (mg/kg bw)				
	0	25	125	500	2000
Total number of animals/sex/group	10	10	10	10	10
Behavioral testing (FOB, motor activity)	10/sex	10/sex	10/sex	10/sex	10/sex
Neuropathology	6/sex	0/sex	0/sex	0/sex	6/sex

3. **Test substance preparation and analysis**: On the day of dosing, each dosing aliquot (including the control article) was sonicated in 40_C water for 5 minutes. After sonication, the aliquots were mixed using a magnetic stir plate and stir bar in a water bath (40_C) until removed for dosing. On sample collection days, the aliquots were mixed with a vortex mixer after sonication but before sample collection. After sample collection, the aliquot was returned to the water bath and maintained there while stirring with a magnetic stir plate until removed for dosing.

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Analysis of the test article concentration in the dose preparations was done by Covance using an analytical method validated by Covance (MP-MT54-MA). The headspace was purged with nitrogen gas until analysis. To determine homogeneity, duplicate samples (1 mL) taken from the top, middle, and bottom of the low- and high-dose preparations were analyzed for test article content. Samples were stored at room temperature until analysis.

To determine stability: one set of samples (1 mL each) was taken from the low- and high-dose levels mixed prestudy and stored in a water bath (40_C) until analyzed. Stability analysis was performed 1) after initial mixing; 2) following re-emulsification 2 days later; and 3) 72 hours after re-emulsification, 5 days after initial mixing. The mixes were reemulsified in a warm water sonicator and vortexed prior to collecting samples. Duplicate samples from the solutions used for dosing were stored in a water bath (40_C) and analyzed on the day of emulsification and at least 72 hours after emulsification.

To determine concentration, duplicate samples from each dose preparation to be administered to test animals were obtained and analyzed. All samples were stored at room temperature until analyzed. Results from the middle homogeneity sample from the low- and high-dose levels are reported as concentration verification.

Results:

Homogeneity analysis: Top, middle and bottom samples ranged from 90-112% of the low dose theoretical values and 111-122% of the high dose theoretical values.

Stability Analysis: For prestudy mixes, values ranged from 79.2-111% for the low-dose level and 111-130% for the high-dose level over 5 days. After at least 72 hours at 40_C storage, mean concentrations of the solutions used for dosing were 93.2 and 112% of the theoretical values of 2.5 and 200 mg/mL, respectively.

Concentration analysis: For duplicate samples, the mean percent of theoretical concentration for the 2.5, 12.5, 50, and 200 mg/mL concentration levels was 110, 128, 114, and 112%, respectively.

The analytical data indicated that the mixing procedure for the 200 mg/mL preparation was adequate, but the 2.5 mg/mL preparation was slightly out of the SOP range for homogeneity. Since there were no adverse effects seen in the homogenous high dose level, the departure from homogeneity for the lower dose had no impact on the study. Dose preparations were stable for 72 hours at 40_C storage at concentrations ranging from 2.5-200 mg/mL. The concentration analysis show that the

preparations were at least as high as the target concentration, and therefore were acceptable.

4. **Statistics**: Levene's test was done to test for variance homogeneity. Transformations were used to stabilize the variance if heterogeneity of variance was at $p \leq 0.05$. One-way ANOVA was used to analyze body weight, body weight change, food consumption, FOB evaluations (continuous data), and motor activity counts. If the ANOVA was significant, Dunnett's multiple comparison t-test was used for control versus treated group comparisons. For each sex, Groups 2-5 were compared with Group 1 (control). Group comparisons were evaluated at the 5%, two-tailed probability level. The reviewer considers the analyses used to be appropriate.

C. **METHODS/OBSERVATIONS**:

1. **Mortality and clinical observations**: Animals were observed twice daily for mortality and morbidity. Cageside observations were made once daily. Detailed clinical observations were recorded once prior to treatment, on Day 1, and weekly thereafter for each animal.

2. **Body weight**: Animals were weighed once prior to treatment and on Days 1, 8, and 15.

3. **Food consumption**: Individual food consumption data were recorded weekly during treatment.

4. **Cholinesterase determination**: Cholinesterase activities were not determined.

5. **Neurobehavioral assessment**:

   a. **Functional observational battery (FOB)**: Each animal underwent a battery of behavioral tests and observations prior to treatment, and on Days 1, 8, and 15 at approximately the same time of day (corresponding to 5 hours post-treatment on Day 1). The observer was blind to treatment status of the animals at the time of testing. As outlined in table below, the FOB consisted of a series of observations of the animals in the cage, during handling, in an open field, and during manipulations to assess reflexes and physiological parameters. Open field observations were recorded for 2 minutes. The auditory response to a Galton whistle was evaluated.

It was not stated whether the same observer was used throughout testing. Neither the environmental conditions nor the test equipment used were described in the study report.

The CHECKED (X) parameters were examined.

X	HOME CAGE OBSERVATIONS	X	HANDLING OBSERVATIONS	X	OPEN FIELD OBSERVATIONS
x	Posture*	x	Reactivity*	x	Mobility
	Convulsions*	x	Lacrimation* / chromodacryorrhea	x	Rearing+
	Tremors*	x	Salivation*		Arousal/ general activity level*
x	Abnormal Movements*	x	Piloerection*		Convulsions*
x	Palpebral closure*	x	Fur appearance		Tremors*
x	Activity	x	Palpebral closure*	x	Abnormal movements*
x	Gait	x	Respiratory rate+	x	Urination / defecation*
	SENSORY OBSERVATIONS		Red/crusty deposits*	x	Grooming
x	Approach response+		Mucous membranes /eye /skin colour	x	Gait abnormalities / posture*
x	Corneal touch response+	x	Eye prominence*	x	Gait score*
x	Startle response*	x	Muscle tone*	x	Bizarre / stereotypic behaviour*
	Pain response*	x	Vocalization	x	Posture
x	Pupil status and response*			x	Time to first step
x	Eyeblink response			x	Locomotor activity
x	Proprioceptive positioning reaction		**PHYSIOLOGICAL OBSERVATIONS**		**NEUROMUSCULAR OBSERVATIONS**
x	Pinna response	x	Body weight*		Hindlimb extensor strength
x	Air righting reflex+	x	Body temperature+	x	Forelimb grip strength*
	Forelimb extension			x	Hindlimb grip strength*
	Olfactory orientation		**OTHER OBSERVATIONS**	x	Landing foot splay*
					Rotarod performance

*Required parameters; +Recommended parameters

b. <u>Locomotor activity</u>: Locomotor activity was evaluated once prior to treatment and on Days 1, 8, and 15 at approximately the same time of day, after the FOB. The animals were placed in an automated photocell activity recording device, and the number of photo beam breaks was recorded for 40 minutes, at 2 minute intervals. Data are summarized as the cumulative activity count over a 10-minute interval.

6. <u>Sacrifice and pathology</u>: At least 15 days following dosing, animals were fasted overnight, then anesthetized with sodium pentobarbital and weighed. Tissues were perfused *in situ* and preserved with 2.0% paraformaldehyde/2.5% glutaraldehyde in 0.1 M phosphate buffer or in

2.0% paraformaldehyde/2.5% glutaraldehyde in a cacodylate solution. Six animals/sex/group in the control and high dose groups were designated for neuropathologic evaluation.

The necropsy included macroscopic examination of the external features of the carcass; external body orifices; external surface of the brain and spinal cord; cervical tissues and organs; thoracic, abdominal, and pelvic cavities and viscera; and nasal cavity and paranasal sinuses. Cut surfaces of the brain and spinal cord were examined when tissue trimming was performed.

The anatomic levels or tissues checked in the table below were trimmed, processed, and embedded in paraffin. Only the tissues from the 6 animals/sex/group designated for neuropathologic evaluations were sectioned and stained with hematoxylin and eosin. The ganglia and nerves checked below were trimmed, processed, and embedded in epoxy resin. Only tissues from those designated for neuropathologic evaluations were sectioned and stained with toluidine blue. Sections of the tissue samples designated for neuropathologic evaluations were sent to Experimental Pathology Laboratories, Inc.

The CHECKED (X) tissues were evaluated.

X	CENTRAL NERVOUS SYSTEM
	BRAIN
x	Forebrain
	Center of cerebrum
x	Midbrain
x	Cerebellum
	Pons
x	Olfactory bulb
x	Medulla oblongata
x	Hypothalamus/thalamus
x	Pituitary gland
x	Caudate nucleus
	SPINAL CORD
x	Cervical swelling
x	Lumbar swelling
x	Thoracic swelling
	OTHER
	Gasserian Ganglion
x	Trigeminal nerves
x	Optic nerve
x	Eyes
x	Gastrocnemius muscle

X	PERIPHERAL NERVOUS SYSTEM
	SCIATIC NERVE
	Mid-thigh
x	Sciatic nerve
	OTHER
x	Sural Nerve
x	Tibial Nerve
	Peroneal Nerve
x	Lumbar dorsal root ganglion
	Lumbar dorsal root fibers
	Lumbar ventral root fibers
x	Cervical dorsal root ganglion
	Cervical dorsal root fibers
	Cervical ventral root fibers
x	Anterior tibialis muscle
x	Macroscopic lesions

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7. **Positive controls**: Positive control data providing evidence of the observational methods to detect major neurotoxic endpoints were provided in two studies. The tests were performed in 1996 by Hazleton Wisconsin, Inc., now Covance Laboratories, Inc. Subcutaneous injections of paraoxon to rats at doses of 0.17 or 0.34 mg/kg bw resulted in dose-related changes in FOB parameters (pinpoint pupils, salivation, tremors, unsteady gait, labored respiration, and hypothermia) and decreases in motor activity when tested at 3 hours postdose (MRID 44163001). Brain cholinesterase was decreased in a dose-related manner. In the second positive control study, rats were injected intraperitoneally with 50 mg/kg bw of acrylamide for 9 or 21 days, at which times an FOB and motor activity tests were conducted (MRID 44163002). The majority of neurotoxic effects were noted on day 21 during the FOB. Compared with controls, treated animals had flaccid muscle tone, gait abnormalities, abnormal extension of hindlimbs, and paralytic hindlimbs. Body weight, grip strength (females), and foot splay were affected and motor activity was decreased. Histopathological changes in the brain and peripheral and spinal cord nerves were apparent only on day 21 and involved Purkinje cell necrosis in the cerebellum and axon degeneration.

   The FOB methods/parameters were similar in the present study (MRID 45932301) and the positive control studies. The equipment used to measure motor activity was not described in the present study, but motor activity tests were of the same session and subsession durations in both groups of studies. The same sections were evaluated for neuropathology, using the same types of stains. Statistical evaluations in the positive control studies were the same as those used in the study being evaluated. The number of animals per test group were the same in the present study and the paraoxon positive control study. Comparative data between two technicians in the paraoxon positive control study showed some differences in inter-observer reliability (different groups of rats were used), but similar overall results. It is unlikely that the same personnel were involved in the positive control studies and the present study, given the 6-year period between studies.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs**: There were no abnormal observations during this study.

2. **Mortality**: All animals survived to the end of the study.

B. **BODY WEIGHT AND BODY WEIGHT GAIN**: There were no effects of MTI-500 on body weight or on body weight gain (Table 2).

TABLE 2. Body weight and body weight gain (g)

Observation	Dose level (mg/kg bw)				
	0	25	125	500	2000
Body weight–Males					
–Day 1	271 ± 32.4	277 ± 39.8	277 ± 26.4	271 ± 30.7	269 ± 31.5
–Day 8	328 ± 36.8	337 ± 40.2	333 ± 28.5	326 ± 36.7	325 ± 39.5
–Day 15	361 ± 38.9	366 ± 39.2	365 ± 30.6	359 ± 40.7	355 ± 40.3
Body weight–Females					
–Day 1	190 ± 17.0	186 ± 20.3	190 ± 18.2	188 ± 12.5	189 ± 18.2
–Day 8	221 ± 19.3	218 ± 19.3	223 ± 17.2	220 ± 14.2	222 ± 21.2
–Day 15	238 ± 18.4	232 ± 21.6	241 ± 17.1	238 ± 18.6	240 ± 20.6
Body weight gain–Males					
–Day 1-8	57 ± 8.4	60 ± 3.9	56 ± 9.7	56 ± 11.3	56 ± 11.5
–Day 8-15	33 ± 5.7	29 ± 4.0	32 ± 5.7	33 ± 6.6	30 ± 4.7
–Day 1-15	90 ± 11.4	89 ± 3.4	88 ± 13.5	89 ± 15.7	86 ± 14.9
Body weight gain–Females					
–Day 1-8	32 ± 4.2	32 ± 5.1	33 ± 6.4	31 ± 4.7	34 ± 7.2
–Day 8-15	16 ± 2.3	15 ± 4.6	18 ± 4.1	19 ± 8.4	18 ± 5.8
–Day 1-15	48 ± 4.0	47 ± 6.9	51 ± 8.3	50 ± 8.9	51 ± 8.2

Data were extracted from MRID 45932301, p.82-83. Values represent mean ± s.d. n=10.
*=p<.05, **=p<.01, when compared to control means.

C. **FOOD CONSUMPTION**: There were no effects of MTI-500 on food consumption.

D. **CHOLINESTERASE ACTIVITIES**: Cholinesterase activities were not measured.

E. **NEUROBEHAVIORAL RESULTS:**

1. **FOB Findings**: There were occasional findings considered representative of normal biologic variation, including: vocalization, stained fur, piloerection, miosis, low and high motor activity, abnormal approach responses, and lack of pupillary response. Females in the 2000 and 500 mg/kg groups had high locomotor activity: 3/10 and 4/10 animals at Day 1 and at Day 15, respectively (Table 3). None of these observations were considered treatment related because the distribution of findings within

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groups had no relation to dose level, the findings were present at similar frequencies in the control group, and/or the finding was present during the prestudy FOB examinations.

No quantitative aspects of the FOB were statistically significant compared to controls, except for a decrease in forelimb grip strength observed during trial 2 on Day 1 in males treated with 125 mg/kg. There were no other grip strength differences noted at any other testing interval and there was no dose dependent increase in the effect; therefore, this statistical significance is not considered biologically meaningful.

FOB recordings were not made for the following required parameters: arousal/general activity levels, convulsions, and tremors.

TABLE 3. Functional observation battery results

Observation	Dose level (mg/kg bw) 0	25	125	500	2000
Males					
Forelimb grip strength (trial 2)					
-Pretest	624 ± 168.0	630 ± 143.9	675 ± 152.8	623 ± 183.4	646 ± 101.8
-Day 1	1295 ± 213.5	1342 ± 254.9	**1063 ± 213.0***	1196 ± 110.0	1293 ± 124.9
-Day 8	1524 ± 248.7	1559 ± 320.4	1440 ± 201.5	1531 ± 148.0	1284 ± 273.0
-Day 15	1665 ± 241.3	1771 ± 302.0	1579 ± 288.5	1734 ± 141.2	1544 ± 209.7
Piloerection (present)					
-Pretest	0/10	0/10	0/10	0/10	0/10
-Day 1	3/10	3/10	4/10	3/10	3/10
-Day 8	2/10	4/10	4/10	5/10	5/10
-Day 15	1/10	2/10	1/10	3/10	2/10
Miosis (present)					
-Pretest	1/10	2/10	1/10	2/10	1/10
-Day 1	2/10	2/10	4/10	5/10	3/10
-Day 8	8/10	2/10	6/10	7/10	5/10
-Day 15	8/10	7/10	6/10	8/10	7/10
Locomotor activity (high)					
-Pretest	0/10	0/10	0/10	0/10	0/10
-Day 1	0/10	0/10	1/10	1/10	1/10
-Day 8	0/10	0/10	1/10	0/10	1/10
-Day 15	0/10	0/10	0/10	0/10	0/10
Females					
Forelimb grip strength (trial 2)					
-Pretest	608 ± 175.8	650 ± 191.3	647 ± 167.8	579 ± 106.9	614 ± 157.6
-Day 1	1132 ± 174.3	960 ± 111.9	1107 ± 112.7	1030 ± 180.7	1166 ± 163.0
-Day 8	1263 ± 188.4	1205 ± 251.7	1272 ± 121.9	1252 ± 140.2	1289 ± 190.3
-Day 15	1289 ± 289.4	1340 ± 191.8	1357 ± 212.2	1447 ± 222.8	1397 ± 145.6
Piloerection (present)					
-Pretest	0/10	0/10	0/10	0/10	0/10
-Day 1	0/10	1/10	0/10	0/10	2/10
-Day 8	0/10	0/10	0/10	1/10	1/10
-Day 15	0/10	0/10	0/10	0/10	0/10

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TABLE 3. Functional observation battery results					
Observation	Dose level (mg/kg bw)				
	0	25	125	500	2000
Miosis (present)					
-Pretest	0/10	0/10	0/10	0/10	2/10
-Day 1	7/10	5/10	7/10	5/10	6/10
-Day 8	6/10	4/10	8/10	7/10	7/10
-Day 15	7/10	7/10	8/10	6/10	9/10
Locomotor activity (high)					
-Pretest	0/10	0/10	0/10	0/10	0/10
-Day 1	1/10	2/10	0/10	1/10	3/10
-Day 8	2/10	1/10	0/10	1/10	1/10
-Day 15	2/10	2/10	0/10	4/10	1/10

Data were extracted from MRID 4593201, pp.50-65; 70-73. Values represent incidence (or other appropriate measure). n=10.
*=p<.05,** p<.01 compared with controls.

2. **Motor activity**: Total motor activity is summarized in Table 4. There were no differences in motor activity data between MTI-500 and control treated groups at any measurement interval. Motor activity declined with each 10 minute subsession, indicating habituation.

TABLE 4. Motor activity (total activity counts for session)					
Test day	Dose level (mg/kg bw)				
	0	25	125	500	2000
Males					
Pre-test	899 ± 146.7	846 ± 267.0	1213 ± 491.8	832 ± 234.5	870 ± 299.3
Day 1	1354 ± 365.8	1098 ± 290.3	1092 ± 371.3	1228 ± 363.5	1251 ± 373.9
Day 8	1465 ± 487.6	1230 ± 735.1	1150 ± 561.8	1556 ± 484.1	1448 ± 577.4
Day 15	1442 ± 408.9	1233 ± 721.1	1157 ± 593.0	1395 ± 591.1	1599 ± 809.8
Females					
Pre-test	874 ± 254.7	1021 ± 561.1	874 ± 294.4	1026 ± 337.9	1153 ± 400.1
Day 1	1338 ± 483.8	1254 ± 307.0	1144 ± 345.7	1387 ± 440.8	1381 ± 478.0
Day 8	1303 ± 611.0	1303 ± 420.1	1097 ± 565.7	1475 ± 642.0	1451 ± 588.4
Day 15	1477 ± 458.6	1286 ± 554.9	1262 ± 580.0	1576 ± 663.7	1498 ± 525.3

Data were extracted from MRID 45932301, p.78-81. Values represent mean ±s.d. n=10

F. SACRIFICE AND PATHOLOGY:

1. **Gross pathology**: There were no treatment related macroscopic changes.

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2. **Brain weight**: Brain weights were not recorded in this study report.

3. **Neuropathology**: There were no treatment-related microscopic changes. The few microscopic observations noted in a few animals in the control group and the highest dose treated group were considered incidental and unrelated to treatment. Because no treatment related findings were noted in the control and highest dose groups, lower dose groups were not tested.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS**: Based on these results, single oral doses of MTI-500 up to the limit dose of 2000 mg/kg produced no neurotoxic effects in rats. The NOAEL for MTI-500 in rats is 2000 mg/kg.

B. **REVIEWER COMMENTS**: Rats were treated to the limit dose of 2000 mg MTI-500/kg without any treatment-related effects. Although a statistically significant decrease in forelimb grip strength was noted, this only occurred in one treatment group (125 mg/kg) at one time point (Day 1) and only in males. Although 3 or more animals (in sample size of 10) were affected in some of the FOB findings, all groups, including the control group, were affected. Females in the two high dose groups (Groups 4 and 5) had high locomotor activity: 3/10 and 4/10 animals in the 2000 mg/kg group at Day 1 and the 500 mg/kg group at Day 15, respectively. Overall, there was no dose- or time-dependence that followed a pattern of treatment-related effects. The few neuropathologic lesions found affected both controls and treated animals and were considered spontaneous findings in the laboratory rat.

Based on the results of this acute oral neurotoxicity study, the NOAEL for MTI-500 (etofenprox) in male and female rats is 2000 mg/kg (limit dose). The LOAEL was not identified (>2000 mg/kg).

C. **STUDY DEFICIENCIES**: Minor deficiencies: The FOB methods do not state whether the same observer was used throughout testing. Neither the environmental conditions nor the test equipment used were described in the FOB study report. FOB recordings were not made for the following required parameters: arousal/general activity levels, convulsions, and tremors. The positive control data are six years old; they should be updated. These deficiencies do not invalidate the study.

DATA FOR ENTRY INTO ISIS

Acute Neurotoxicity Study - rats (870.6200a)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
128965	45932301	acute neurotox	rats	1 dose	oral	gavage	25-2000	0, 25, 125, 500, 2000	2000	not determined	none	

DATA EVALUATION RECORD

ETOFENPROX (MTI-500)/128965
[OPPTS 870.6200b (§82-7)]

STUDY TYPE: SUBCHRONIC NEUROTOXICITY
MRID 45925701

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 18-2004

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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EPA Reviewer: Meta Bonner, Ph.D. **Signature:** M. Tag. Bo
Registration Action Branch 3, Health Effects Division (7509C) **Date** May 10, 2
EPA Secondary Reviewer: Gerome Burke, Ph.D. **Signature:**
Registration Action Branch 3, Health Effects Division (7509C) **Date** June 1, 20
EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:**
Registration Action Branch 3, Health Effects Division (7509C) **Date** 6/1/06

Template version 11/01

TXR#: 0052279

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity [OPPTS 870.6200b (§82-7)] - feeding, rat; (No OECD guideline).

PC CODE: 128965 **DP BARCODE:** D297157
SUBMISSION NO.: Not available

TEST MATERIAL (PURITY): MTI-500 (Etofenprox, 99%)

SYNONYMS: Etofenprox, Ethofenprox , RF 316 , SAN 811 I , Zoecon RF-316 , 2-(4-Ethoxyphenyl)-2-methylpropyl 3-phenoxybenzyl ether, Benzene, 1-((2-(4-ethoxyphenyl)-2-methylpropoxy)methyl)-3-phenoxy- (9CI) (CA INDEX NAME)

CITATION: Smith, Peter B. (2003) 13-Week dietary neurotoxicity study with MTI-500 in rats. Covance Laboratories Inc., 3301 Kinsman Boulevard, Madison, WI 53704-2595. Covance 6648-153, April 7, 2003. MRID 45925701. Unpublished.

SPONSOR: Mitsui Chemicals, Inc., 3-2-5, Kasumigaseki, Chiyoda-ku, Tokyo 100-6070, Japan.

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study (MRID 45925701) MTI-500 (etofenprox, 99% a.i., lot #87031) was administered to 10 Crl:CD®(SD)IGS BR rats/sex/group at dose levels of 0, 2500, 5000, or 10,000 ppm in the diet (equivalent to 0, 149, 299, or 604 mg/kg bw/day for males and 0, 174, 350, or 690 mg/kg/day for females) for 13 weeks. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed in 10 animals/sex/group pretreatment and at 1, 5, 9, and 13 weeks. Cholinesterase activities were not determined. At study termination, 10 animals/sex/group were euthanized and perfused in situ for neuropathological examination. Of the perfused animals, six animals/sex from the control and 10,000 ppm group were subjected to histopathological evaluation of brain and peripheral nervous system tissues. Brains were not weighed.

All animals survived to terminal sacrifice. Body weight and body weight gain were affected only in females. The final body weight of females in the 690 mg/kg/day group was 89% of the control group weight (NS). For females, body weight gains were reduced by 16-29% in all treated groups (all $p<0.05$). The lower body weight gains in females correlated with a slightly lower

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total food consumption in all treated female groups (87-92% of the control value). During the FOB, an abnormal tiptoe gait was observed in all female groups week 5 to week 13 (control 1/10, low-dose 3/10, mid-dose 5/10, high-dose 3/10). This trait was not observed in males. During the FOB, brown stained fur was observed in some males and females of all groups by week 5. The number of animals affected was generally higher in the treated groups, but the numbers were not dose related. Although possibly treatment related, the finding of stained fur was not considered adverse. Increased rearing was noted in all treated males at 1-week (at least 4-fold for the lower doses and double for the highest dose), only statistically significant for the low- and mid-dose treated males. Increased rearing behavior did not occur at any other time point for males and was not observed for females. There were no treatment related effects on neuropathology. Liver weight were increased in both sexes, both absolute and relative at the high dose (27- 42%), at the mid dose (16-22%) only in males, and only for the relative measure at the low dose (13%) in males. Liver histology was not assessed.

Based on the results of this subchronic neurotoxicity study, the NOAEL for MTI-500 (etofenprox) in rats was not established. The LOAEL is 149 mg/kg/day based on increased liver weights in males, abnormal tiptoe gait in females and increase rearing behavior in males.

The study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for a subchronic neurotoxicity study in rats (870.6200b). Minor deficiencies include the following: The FOB methods do not state whether the same observer was used throughout testing; neither the environmental conditions nor the test equipment used in the FOB and motor activity testing were described; FOB recordings were not made for some required parameters; and the positive control data are from the year 1996 and should be updated.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. A flagging statement was not provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: MTI-500

Description:	off-white to light yellow solid
Lot #:	87031
Purity:	99% a.i.
Compound Stability:	stable for at least 15 days at room temperature (expiration date: December 2003)
CAS # of TGAI:	80844-07-1
Structure:	Not given in the study report

2. Vehicle and/or positive control: The test material was liquified and mixed with corn oil.

3. Test animals:

Species:	rat	
Strain:	Crl:CD®(SD)IGS BR	
Age/weight at study initiation:	54-60 days old males: 242-306 g; females: 168-233 g	
Source:	Charles River Laboratories, Portage, MI	
Housing:	individually, in suspended, stainless-steel cages	
Diet:	Harlan Teklad (Madison. WI) certified rodent diet (#8728CM), *ad libitum*	
Water:	Source not described, *ad libitum*	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	30-70%
	Air changes:	10/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	16 days	

B. STUDY DESIGN:

1. In life dates: Start: June 5, 2002; End: September 9, 2002

2. Animal assignment and treatment: Animals were stratified by pre-exposure body weight and then randomly assigned via a computer program to the test groups noted in Table 1. Test substance was administered for 13 weeks. Dose levels were chosen based a 28-day range-finding study. In the 28-day study, a concentration of 10,000 ppm was well tolerated with a slight effect on weight gain. Therefore, 10,000 ppm was chosen as the highest concentration in the present study. Lower concentrations were based on fractions of the upper concentration.

TABLE 1. Study design

	Dose group (ppm in diet)							
	Control (0 ppm)		Low dose (2500 ppm)		Mid dose (5000 ppm)		High dose (10,000 ppm)	
Experimental parameter	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Mean dose to animal (mg/kg/day)	0	0	149	174	299	350	604	690

Total number of animals/group	10	10	10	10	10	10	10	10
Behavioral testing (FOB, Motor Activity)	10	10	10	10	10	10	10	10
Neuropathology	6	6	—	—	—	—	6	6

Data taken from pp. 16 and 23, MRID 45925701.

3. **Test substance preparation and analysis:** The test material was liquified by purging the test material container with nitrogen gas and then heating the container in an incubator set at approximately 60°C. The required amount of liquified test material, corrected for 99% purity, was then weighed into a container and the required amount of corn oil was added. The test material was evenly disbursed in the corn oil by stirring with a spatula. Diet was prepared approximately biweekly by mixing appropriate amounts of the test substance/corn oil with Harlan Teklad Certified Rodent Diet #8728CM. A premix was first formed; additional amounts of diet were used to rinse the test material container. Total mixing time in a Hobart mixer (H-600T) was at least 15 minutes. Each dose level was prepared independently in sequential order of increasing concentration. The diets were stored at room temperature in labeled containers. Homogeneity was tested at week 1-2 by taking samples from the top, middle, and bottom of the low- and high-dose preparations and analyzing in duplicate. During weeks 1-2, 3-4, 7-8, and 13-14 of the study, samples of control and treated food at all dose levels were analyzed for concentration. A stability study was conducted independent of the present study (Covance 6648-152).

Results:

Homogeneity analysis: Concentrations in samples from the top, middle and bottom of the low-dose mixture ranged from 90.0-92.4% of the target concentration (mean, 91.4%). The relative standard deviation was 1.1%. Concentrations in samples from the high-dose mixture ranged from 92.7-96.1% of the target concentration (mean 94.8%). The relative standard deviation was 1.3%.

Stability analysis: Dietary mixtures were reported to be stable for at least 15 days when stored at room temperature (Covance 6648-152).

Concentration analysis: Concentrations in the 2500, 5000, and 10,000 ppm mixtures over the four sample periods ranged from 86.4-92.8%, 89.2-92.3%, and 85.9-95.2% of the target concentration. Mean concentrations of all groups ranged from 90.2-91.8%.

Concentrations were slightly low, but all mean values were within 10% of the target concentrations. Thus, the analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. **Statistics:** Variance homogeneity was analyzed with Levene's test. For heterogeneity of variance, Draper and Hunter transformations were used to stabilize the variance. Body weight, body weight changes, food consumption, FOB evaluations (continuous data), motor activity counts, clinical pathology data (continuous), and organ weight data were analyzed by one-way analysis of variance (ANOVA). If the ANOVA was significant, Dunnett's multiple comparison was used for control versus treated group comparisons. Each treated group, by

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sex, was compared with the respective control group. Significance was flagged at $p<0.05$. The Reviewer considers the analyses used to be appropriate.

C. METHODS/OBSERVATIONS:

1. **Mortality and clinical observations:** Animals were observed twice daily for mortality and moribundity. Detailed clinical observations were recorded weekly.

2. **Body weight:** Animals were weighed weekly.

3. **Food consumption:** Individual food consumption was measured daily and recorded weekly. Test substance intake was calculated from the dietary test material concentration, food consumption, and body weight data.

4. **Cholinesterase determination:** Cholinesterase activities were not determined.

5. **Neurobehavioral assessment:**

   a. **Functional observational battery (FOB):** Behavioral testing was performed pretreatment and during weeks 1, 5, 9, and 13. The FOB included observations of animals in the cage, during handling, in an open arena (for 2 minutes), and during manipulations to assess reflexes and physiological parameters. Where applicable, findings were ranked according to the degree of severity. The observer who conducted the FOB was blind to the animal's treatment group. The time of testing and environmental conditions were not described. Testing equipment was not described.

The CHECKED (X) parameters were examined.

X	HOME CAGE OBSERVATIONS	X	HANDLING OBSERVATIONS	X	OPEN FIELD OBSERVATIONS
X	Posture*	X	Reactivity*		Mobility
	Biting	X	Lacrimation* / chromodacryorrhea	X	Rearing+
	Convulsions*	X	Salivation*		Arousal/ general activity level*
X	Tremors* (unusual behavior)	X	Piloerection*	X	Convulsions*
X	Abnormal Movements*	X	Fur appearance	X	Tremors*
	Palpebral closure*	X	Palpebral closure*	X	Abnormal movements*
	Faeces consistency	X	Respiratory rate+	X	Urination / defecation*
X	Activity		Red/crusty deposits*	X	Grooming
	SENSORY OBSERVATIONS		Mucous membranes /eye /skin colour	X	Gait abnormalities / posture*
X	Approach response+	X	Eye prominence*		Gait score*
	Touch response+	X	Muscle tone*	X	Bizarre / stereotypic behaviour*
X	Startle response*	X	Vocalizations		Backing
X	Pain response*			X	Time to first step
X	Pupil response*				
	Eyeblink response		**PHYSIOLOGICAL OBSERVATIONS**		**NEUROMUSCULAR OBSERVATIONS**
	Forelimb extension	X	Body weight*		Hindlimb extensor strength

X	Hindlimb extension	X	Body temperature+	X	Forelimb grip strength*
X	Air righting reflex+			X	Hindlimb grip strength*
	Olfactory orientation			X	Landing foot splay*
			OTHER OBSERVATIONS		Rotarod performance

*Required parameters; +Recommended parameters

b. **Motor activity:** Motor Activity was evaluated following the FOB. The sessions were 40 minutes (four 10-minute subsessions). An automated photocell activity recording device was used (the brand was not named). Only total motor activity was measured; locomotor activity was not evaluated.

6. **Sacrifice and pathology:** Following overnight fasting, animals were bled for clinical chemistry tests (not required for subchronic neurotoxicity studies) and anesthetized with sodium pentobarbital. All animals were perfused *in situ* with 2% paraformaldehyde/2.5% glutaraldehyde in 0.1 M phosphate buffer, but tissues from only six animals/sex from the control and high-dose group were examined. The liver and thyroid with parathyroid were weighed and preserved in formalin. The brain was not weighed. Tissues (with the exception of the peripheral nerves and ganglia) were embedded in paraffin, sectioned (thickness not stated), and stained with hematoxylin and eosin. The cervical and lumbar dorsal root ganglia, trigeminal ganglion, and optic, sciatic, tibial, and sural nerves were embedded in glycol methacrylate, sectioned (thickness not stated), and stained with toluidine blue. Several sections were made through most tissues. Additional examined tissues and sections are listed in Table 29 and Appendix 7 of the study report.

The CHECKED (X) tissues from the control and high-dose group were evaluated.

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Forebrain	X	Mid-thigh (area not described)
X	Center of cerebrum		Sciatic Notch
X	Midbrain		
X	Cerebellum		**OTHER**
X	Pons	X	Sural Nerve
X	Medulla oblongata	X	Tibial Nerve
	SPINAL CORD		Peroneal Nerve
X	Cervical swelling	X	Lumbar dorsal root ganglion
X	Lumbar swelling		Lumbar dorsal root fibers
X	Thoracic swelling		Lumbar ventral root fibers
	OTHER	X	Cervical dorsal root ganglion
	Gasserian Ganglion		Cervical dorsal root fibers
X	Trigeminal nerves		Cervical ventral root fibers
X	Optic nerve		
X	Eyes		
X	Gastrocnemius muscle		

7. **Positive controls:** Positive control data providing evidence of the observational methods to detect major neurotoxic endpoints were provided in two studies. The tests were performed in 1996 by Hazleton Wisconsin, Inc., now Covance Laboratories, Inc. Subcutaneous injections of paraoxon to rats at doses of 0.17 or 0.34 mg/kg bw resulted in dose-related changes in FOB parameters (pinpoint pupils, salivation, tremors, unsteady gait, labored respiration, and hypothermia) and decreases in motor activity when tested at 3 hours postdose (MRID 44163001). Brain cholinesterase was decreased in a dose-related manner. In the second positive control study, rats were injected intraperitoneally with 50 mg/kg bw of acrylamide for 9 or 21 days, at which times an FOB and motor activity tests were conducted (MRID 44163002). The majority of neurotoxic effects were noted on day 21 during the FOB. Compared with controls, treated animals had flaccid muscle tone, gait abnormalities, abnormal extension of hindlimbs, and paralytic hindlimbs. Body weight, grip strength (females), and foot splay were affected and motor activity was decreased. Histopathological changes in the brain and peripheral and spinal cord nerves were apparent only on day 21 and involved Purkinje cell necrosis in the cerebellum and axon degeneration.

   The FOB methods/parameters were similar in the present study (MRID 45932701) and the positive control studies. The equipment used to measure motor activity was not described in the present study, but motor activity tests were of the same session and subsession durations in both groups of studies. The same sections were evaluated for neuropathology, using the same types of stains. Statistical evaluations in the positive control studies were the same as those used in the study being evaluated. The number of animals per test group were the same in the present study and the paraoxon positive control study. Comparative data between two technicians in the paraoxon positive control study showed some differences in inter-observer reliability (different groups of rats were used), but similar overall results. It is unlikely that the same personnel were involved in the positive control studies and the present study, given the 7-year period between studies.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs:** No clinical signs clearly related to treatment were observed. Barbering (self-plucking of the hair in various regions of the body) was observed in all dose groups. Incidences (total animals involved) were 1, 4, 1, and 3 males in the control, 2500, 5000, and 10,000 ppm groups, respectively. This action was observed in 1, 2, 3, and 5 females in the control, 2500, 5000, and 10,000 ppm groups, respectively. In some cases more than one body region was involved (i.e., front paw, hindlimb). This observation was first recorded in several animals on day 36.

2. **Mortality:** All animals survived to the end of the study.

B. BODY WEIGHT AND BODY WEIGHT GAIN:

Body weight and body weight gain values for males and females are summarized in Table 2. Body weight of males was unaffected by treatment with MTI-500. Final body weights of males in the treated groups were 97-98% of the control body weight. The final mean body weight of females that received 10,000 ppm in the diet was 89% of the control value (NS). Only the day 50 value

was statistically significantly lower than the control value, 255g vs 285 g ($p<0.05$). Body weight in the other female groups was unaffected by treatment. In males, body weight gains were unaffected by treatment; the final weight gain in the 10,000 ppm group was 93% of the control weight gain. In females, weight gains in all treated groups were lower (by 43-57%) than the control gain during the first week of treatment (all $p<0.05$) (data not shown). Values were similar thereafter, but the gains of treated female groups remained 16-29% below that of the control group at terminal sacrifice (all $p<0.05$).

TABLE 2. Body weight and body weight gain (g ± s.d.)

Observation	Dose level (ppm)			
	Control (0 ppm)	Low dose (2500 ppm)	Mid dose (5000 ppm)	High dose (10,000 ppm)
Body weight–Males				
– *Day #1*	275±17.2	274±16.4	273±14.4	275±17.5
– *Day #43*	456±37.8	443±32.4	433±29.9	438±26.2
– *Day # 92*	528±53.6	519±43.9 (98%)	510±40.6 (97%)	512±40.2 (97%)
Body weight–Females				
– *Day #1*	200±15.5	197±17.3	198±20.2	198±17.5
– *Day #43*	282±22.9	261±23.6	261±18.8	257±27.5
– *Day #92*	300±27.7	281±27.7 (94%)	280±26.3 (93%)	268±24.1 (89%)
Body weight gain–Males				
– *Days* #1-92	253±39.8	245±43.1 (97%)	237±34.4 (94%)	236±29.6 (93%)
Body weight gain–Females				
– *Days* #1-92	100±17.4	**84*±17.5** (84%)	**82*±11.5** (82%)	**71*±11.5** (71%)

Data were extracted from Tables 22 and 23, pp. 105-112, MRID 45925701.
Values represent mean ± s.d.; values in parentheses are percent of control, calculated by reviewer.
n=10.
*=$p<0.05$, when compared to control means.

C. **FOOD CONSUMPTION**: Weekly and total food consumption were unaffected by treatment in males (Table 3). Females in all treatment groups, but primarily the high-dose group, showed lower food consumption values during weeks 1, 4, 5, 9, 10, and 11 (all $p<0.05$). Weekly values in the 10,000 ppm group ranged from 77-96% of the respective control values. The total food consumed by females in the 10,000 ppm group was 87% of the control value. Food consumption was slightly lower in the 2500 and 5000 ppm groups, 91 and 92% of the control value, respectively.

Food efficiencies of the control, low-, mid-, and high-dose female groups, calculated by the Reviewer [(body weight gain/food consumption) x 100], were 5.7, 5.3, 5.1, and 4.7, respectively (93, 89, and 82% of the control value).

TABLE 3. Food consumption (total g)

Week No.	Dose level (ppm)			
	Control (0 ppm)	Low dose (2500 ppm)	Mid dose (5000 ppm)	High dose (10,000 ppm)
Males				
-Days #1-92	2353	2292 (97%)	2256 (96%)	2330 (99%)
Females				
-Days #1-92	1742	1586 (91%)	1611 (92%)	1509 (87%)

Data were extracted from Table 24, pp. 113-116, MRID 45925701.
Values represent means (standard deviations were not calculated).
Values in parentheses are percent of control, calculated by reviewer.
n=4-9.

D. CHOLINESTERASE ACTIVITIES: Cholinesterase activities were not measured.

E. NEUROBEHAVIORAL RESULTS:

1. **FOB Findings:** In the open field, an abnormal gait (described as tiptoe) was observed in two females in the 5000 ppm group during week 1; in 1, 1, 3, and 1 females in the control through high-dose groups during week 5; in 1, 3, 4, and 1 females in the control through high-dose groups during week 9; and in 1, 3, 5, and 3 females in the control though high-dose groups during week 13 (Table 4). The observations were consistently seen in the same animals. Red- or brown-stained fur was also observed, primarily in females, during weeks 5, 9, and 13. Incidences in the treated groups were higher than in the control group (the numbers in Table 4 refer to number of animals; some animals exhibited stained fur in more than one body area). During week 13, the total number of females involved was 1, 4, 4, and 4 in the control through high-dose groups. The brown stain was not further described, but it was observed primarily on the front limbs and shoulders. Occasionally, a red stain was found on the head and dorsal cervical area. Other significant differences between MTI-500 treated groups and the controls were sporadic and not dose related, e.g., increased number of rears for males fed 2500 and 5000 ppm during week 1.

   FOB recordings were not made for some required parameters: Home Cage Observations - convulsions and palpebral closure, Handling Observations - red/crusty deposits, Open Field Observations - arousal/general activity levels and gait score.

TABLE 4. Functional observational battery results

Observation	Dose level (ppm in diet)			
	Control (0 ppm)	Low dose (2500 ppm)	Mid dose (5000 ppm)	High dose (10,000 ppm)
Males				
Abnormal gait (tiptoe)				
Pretest	0	0	0	0
Week 1	0	0	0	0
Week 5	0	0	0	0
Week 9	0	0	0	0

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TABLE 4. Functional observational battery results				
	Dose level (ppm in diet)			
Observation	**Control (0 ppm)**	**Low dose (2500 ppm)**	**Mid dose (5000 ppm)**	**High dose (10,000 ppm)**
Week 13	0	0	0	0
Stained fur				
Pretest	0	0	0	0
Week 1	0	1	0	0
Week 5	1	2	0	0
Week 9	1	2	0	1
Week 13	1	2	0	3
Rearing				
Week 1	2.4 ± 2.80	**9.3 ± 6.15***	**10.3 ± 6.96***	5.8 ± 5.53
Females				
Abnormal gait (tiptoe)				
Pretest	0	0	0	0
Week 1	0	0	2	0
Week 5	1	1	3	1
Week 9	1	3	4	1
Week 13	1	3	5	3
Stained fur				
Pretest	0	0	0	0
Week 1	0	0	0	0
Week 5	0	2	2	2
Week 9	0	2	3	3
Week 13	1	4	4	4
Rearing				
Week 1	14.3 ± 8.77	11.0 ± 7.45	13.4 ± 9.86	17.0 ± 6.96

Data were extracted from Tables 4-8, pp. 59-82, Table 10, page 84, and Appendix 2, pp. 179-477, MRID 45925701.
Values represent number of affected animals. n=10. *=p<.05, **=p<.01, when compared to control means.

2. **Motor activity**: Total motor activity over 40 minutes is summarized in Table 5. There was no affect of treatment on motor activity of males or females at any time point. None of the subsessions were statistically significant. Habituation was reached by controls and treated groups by the third 10-minute subsession (at approximately 24 minutes).

TABLE 5. Motor activity (total activity counts for 40-minute session)				
	Dose level (ppm in diet)			
Test day	**Control (0 ppm)**	**Low dose (2500 ppm)**	**Mid dose (5000 ppm)**	**High dose (10,000 ppm)**
Males				
Pre-test	1253±500	1567±382	1445±469	1477±323
Week 1	1377±432	1421±387	1380±510	1430±294
Week 5	1649±583	1575±455	1813±405	1682±261
Week 9	1614±326	1453±436	1491±495	1622±449

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TABLE 5. Motor activity (total activity counts for 40-minute session)				
	Dose level (ppm in diet)			
Test day	Control (0 ppm)	Low dose (2500 ppm)	Mid dose (5000 ppm)	High dose (10,000 ppm)
Week 13	1461±566	1538±545	1156±720	1641±365
Females				
Pre-test	1459±467	1594±461	1552±526	1246±519
Week 1	1380±406	1563±453	1564±323	1213±517
Week 5	1666±435	1885±482	1823±349	1411±547
Week 9	1782±362	1859±378	1654±525	1390±432
Week 13	1414±555	1825±305	1579±440	1388±544

Data were extracted from Table 19, pp. 97-102, MRID 45925701.
Values represent mean ±s.d. n=10.

F. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** There were no treatment-related macroscopic lesions in males or females. In males, absolute liver weights in the 5000 and 10,000 ppm groups were increased over controls by 16 and 27%, respectively (both $p<0.05$), and relative liver weights in all treated male groups were increased by 13-30% in a dose-related manner (all $p<0.05$) (Table 6). In females, absolute and relative liver weights were increased by 29 and 42%, respectively, in the 10,000 ppm group (both $p<0.05$). There was no effect of treatment on absolute or relative thyroid/parathyroid weight in either sex. Liver and thyroid weights are not required for neurotoxicity studies. Ophthalmic examination found no visible lesions.

TABLE 6. Liver weights (absolute and relative to body weight in mg)				
	Dose level (ppm in diet)			
Sex	Control (0 ppm)	Low dose (2500 ppm)	Mid dose (5000 ppm)	High dose (10,000 ppm)
Males - absolute	13.02 ± 1.21	14.47 ± 1.76	**15.06 ± 1.59*** (↑16%)	**16.49 ± 2.61*** (↑27%)
Males - relative	2.62 ± 0.28	**2.97 ± 0.22*** (↑13%)	**3.19 ± 0.26*** (↑22%)	**3.41 ± 0.32*** (↑30%)
Females -absolute	9.14 ± 0.80	8.83 ± 1.49	9.57 ± 1.18	**11.77 ± 2.97*** (↑29%)
Females - relative	3.30 ± 0.34	3.42 ± 0.52	3.69 ± 0.41	**4.7 ± 1.0*** (↑42%)

Data were extracted from Table 27, pp. 127-128, MRID 45925701. *=p<.05, **=p<.01, when compared to control means.
Values represent mean ±s.d. n=10.

2. **Brain weight:** Brains were not weighed.

3. **Neuropathology:** No treatment-related lesions were observed in males or females. The few microscopic lesions observed in the 0 and 10,000 ppm groups are considered incidental to treatment. These include degeneration of the trigeminal axon in two males in the control group and 3 males in the 10,000 ppm group and pituitary cysts or Rathke's pouch remnants in

two males in each group. Incidences of axonal degeneration of peripheral nerves (tibial, sural), dorsal root ganglia, and spinal nerves and spinal cord white areas (all graded minimal) were generally similar between the 0 and 10,000 ppm groups for each sex. Inflammation of the gastrocnemius muscle was observed in one male in the control group, and myofiber degeneration of the anterior tibialis muscle was observed in one male in the 10,000 ppm group.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The investigators did not consider the 11% reduction of final body weight in females and the stained fur observed in males and females of all treated groups adverse neurotoxic effects. There were no effects of treatment on other FOB parameters, motor activity, or neuropathology. Therefore, in the absence of neurotoxic effects, they considered the no-adverse-effect dose to be 604 mg/kg/day for males and 690 mg/kg/day for females.

B. REVIEWER COMMENTS: Administration of MTI-500 in the diet at concentrations up to 10,000 ppm for 91 days did not result in clinical signs or changes in FOB parameters or motor activity that were clearly treatment related. Self-barbering was observed after one month of treatment and incidences were dose-related in females. The study authors state that this behavior was not observed in an earlier 4-week study (it was observed in the present study only after a month of treatment). However, the toxicological significance of self-barbering is questionable. Abnormal gait (tiptoe), observed only in females during the FOB was not dose-related. Stained fur, also observed during the FOB, was not dose-related in either males or females, but by week 13, incidences were higher in all treated female groups compared with the female control group. The stained fur was not further described, and in the absence of other neurotoxicological effects is of questionable toxicological significance. A check of the animals involved indicated that self-barbering and stained fur occurred in different animals. The mean final body weight of females that received 10,000 ppm for 91 days was 89% of the control value; body weights of females in the other treated groups were only slightly affected (93-94% of the control value). Body weights of males in all treated groups were unaffected. Body weight gains of females in all treated groups were significantly lower by 16-29% ($p<0.05$) than the female control group gain. In association with the reduced body weight gains, total food consumption was reduced by 8-13% in all treated female groups and food efficiency was reduced in the 10,000 ppm group (82% of the control value). In the absence of obvious neurological effects on FOB parameters and motor activity, the reduced body weight gains in all treated female groups and the 11% body weight reduction in females that received 10,000 ppm are not considered adverse. The Reviewer agrees with the study authors concerning the NOAEL and LOAEL. Although a limit dose was not achieved, the 11% lower body weight of females receiving 10,000 ppm in the diet indicates that a limit dose was approached.

Based on the results of this subchronic neurotoxicity study, the NOAEL for MTI-500 (etofenprox) in rats was not established. The LOAEL is 149 mg/kg/day based on increased liver weights in males, abnormal tiptoe gait in females and increase rearing behavior in males.

C. **STUDY DEFICIENCIES:** Minor deficiencies: The red and brown staining of fur should have been further described. There was no indication to the Reviewer whether the stain was due to blood, fecal matter, or browsing through the oily food. FOB recordings were not made for some required parameters: Home Cage Observations - convulsions and palpebral closure, Handling Observations - red/crusty deposits, Open Field Observations - arousal/general activity levels and gait score. The time of testing, environmental conditions, and equipment used for measurement of FOB parameters (strain gage, photocell type) were not described. The positive control data are seven years old; they should be updated.

DATA EVALUATION RECORD

ETOFENPROX

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT; OPPTS 870.6300

MRID 46062301

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
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Task No. 18-2004

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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EPA Reviewer: M. Bonner, Ph.D. **Signature:**
Registration Action Branch 3, Health Effects Division (7509C) **Date** 5-15-20
EPA Secondary Reviewer: K. Raffaele, Ph.D. **Signature:**
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EPA Work Assignment Manager: M. Ottley, Ph.D. **Signature:**
Registration Action Branch 3, Health Effects Division (7509C) **Date** 6/1/06

TXR#: 0052279

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat
[OPPTS 870.6300 (§83-6)] OECD 426

PC CODE: 128965 **DP BARCODE:** D297157
SUBMISSION NO.: none

TEST MATERIAL (PURITY): Etofenprox Technical (99.2%)

SYNONYMS: 2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzyl ether

CITATION: Myers, D.P. (2003) Etofenprox developmental neurotoxicity study in the rat by oral (dietary) administration. Huntingdon Life Sciences Ltd., Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England. Laboratory project id. MTU 215/032731; August 7, 2003. MRID 46062301. Unpublished

SPONSOR: Mitsui Chemicals, Inc., 1144, Togo, Mobara, Chiba, 297-0017, Japan

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46062301), Etofenprox technical (99.2% a.i., batch # 87137) was administered to 24 female Crl:CD® (SD)BR IGS rats/dose in the diet at concentrations of 0, 250, 700 or 2100 ppm from gestation day (GD) 6 through postnatal day (PND) 21. The average daily test article intake was 0, 21, 57, and 169 mg/kg/day during gestation. Previously submitted data (MRID 40449723) showed that the etofenprox is present in rat milk. A Functional Operational Battery (FOB) was performed on 10 dams/dose on GDs 12 and 18, and on lactation days 4, 11, and 21. On postnatal day 4, litters were culled to yield four males and four females (as closely as possible). Offspring representing at least 20 litters/dose were allocated for detailed clinical observations (FOB), assessment of motor activity, assessment of auditory startle response

habituation, assessment of auditory startle pre-pulse inhibition, assessment of learning and memory, and neuropathology at study termination (day 63-67 of age). On postnatal day 21, the whole brain was collected from 10 pups/sex/dietary level for micropathologic examination and morphometric analysis. Pup sexual maturation was assessed by age at vaginal opening for females and age at completion of balano-preputial separation for males.

For mothers, no treatment-related effects were observed on survival, body weight, food consumption or clinical signs. Body weight gains were lower (14%) in the mid- and high-dose groups at interval days 6-10 during gestation, and were higher (21- 61%) in the high-dose group for the lactation period (days 1-21). Following current policy the increase body weight gain during lactation was not considered as an adverse finding for this study. The FOB incidence of rearing behavior was increased (129%) in high-dose females. **The maternal LOAEL is 2100 ppm (169 mg/kg/day, HDT) based on increased incidence of rearing behavior in the FOB. The maternal NOAEL is 700 ppm (57 mg/kg/day).**

Treatment had no adverse effects on survival, body weight, body weight gain, developmental landmarks, learning and memory, brain weights, brain morphology, or neuropathology.

Treatment-related clinical signs of toxicity in the offspring included eye lesions and body injuries. Combinations of one or both eyes large/prominent, dark, and opaque were observed in one control pup, two pups from two low-dose litters, five pups from three mid-dose litters and in twelve pups from nine high-dose litters. Five of these high-dose pups were killed between days 18-28 and one mid-dose pup was killed on day 31 for reasons of animal welfare. Ophthalmoscopic examination of some of the affected pups revealed unilateral intraoccular hemorrhage. In addition, tail and paw injuries or trauma such as cuts, bleeding, swelling, redness, or bruises were seen on a total of 11 pups from four high-dose litters and on 5 pups from three mid-dose litters compared with only 1-2 findings in one litter each of the low-dose and control groups. It is possible that the eye observations are in part or wholly resulting from altered maternal behavior due to treatment, particularly considering the body injuries also observed for the pups.

No treatment-related effects on overall or interval motor or locomotor activity were seen in female offspring at any dose level on any test day or in male offspring at the low and mid dose groups at any test day. In the high

dose males, however, there was a statistically significant lower motor activity level than controls on PND 58.

During auditory startle habituation testing, high-dose males and females had greater peak amplitudes than controls for nearly all trial blocks on both testing days, and latency to peak response was lower for high-dose males and females compared with the controls on PND 58. For pre-pulse inhibition on PND 58, high-dose males showed a greater inhibition of response and a longer latency than the control group.

The offspring LOAEL is 700 ppm (57 mg/kg/day) based on eye abnormalities in both sexes. The offspring NOAEL is 250 ppm (21 mg/kg/day). (Note however that observations of eye injury may be the result of altered material care of the pups and further note that pup behavior was altered for auditory startle in both sexes at the high dose.)

This study is classified **acceptable/non-guideline** developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft). This study could be considered ungradable to guideline study if the deficiencies are addressed. The analytical data for homogeneity and stability analysis were not provided. This study is also considered non-guideline at this time due to deficiency in the memory assessment in the offspring, lack of descriptions for behavioral equipment used, and pending a comprehensive review of all available positive control data.

COMPLIANCE: Signed and dated Flagging, GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Etofenprox Technical

Description:	white solid, amber liquid when molten
Lot/Batch #:	87137
Purity:	99.2 % a.i.
Compound Stability:	documented by sponsor; used before expiry date of Dec. 2003
CAS # of TGAI:	80844-07-1

2. **Vehicle and/or positive control:** The test article was stirred into corn oil prior to adding to the diet.

3. **Test animals (P):**

Species:	Rat	
Strain:	Crl:CD® (SD)BR IGS	
Age at study initiation:	9-10 weeks	
Wt. at study initiation:	199-361.0 g	
Source:	Charles River UK Limited	
Housing:	Individually or with litter in stainless steel grid or plastic cages	
Diet:	UAR VRF1 Certified powdered rodent diet, *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	Temperature:	19-23°C
	Humidity:	40-70%
	Air changes:	15/hour
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 1 week	

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: August 28, 2002; End: December 4, 2002

2. **Study schedule:** The maternal animals were mated and assigned to study. The test substance was administered to the maternal animals (24/dose group) from gestation day 6 through lactation day 21. Pups were weaned onto control diets on postnatal day 21, after which time maternal animals were killed. F_1 pups remained on study up to about postnatal day 65.

3. **Mating procedure**: Females were paired 1:1 with males of the same strain and source. Mating was determined by the observation of ejected copulation plugs and/or the presence of sperm in a vaginal smear. The day evidence of mating was found was designated gestation day 0.

4. **Animal assignment**: Mated females were allocated in sequence to the experimental groups shown in Table 1. For offspring, six sets of animals were utilized for assessment at each age (Table 1). Generally one male or one female offspring from each litter was allocated on PND 4 to each of 5 different functional investigations and a further male and female pup from each litter was allocated for sacrifice and brain examinations on PND 21.

   A minimum of ten pups/sex/group were allocated on postnatal day 4 to each of the following: motor activity, auditory startle habituation, auditory startle inhibition, learning and memory, detailed observational battery, and sacrifice and brain examination on postnatal day 21. Cholinesterase activities in blood and brain were not measured.

TABLE 1. Study design

Experimental parameter		Dietary concentration (ppm) 0	250	700	2100
Maternal animals					
		No. of maternal animals assigned			
FOB (GD 12, 18; LD 4, 11, 21)		10	10	10	10
Offspring					
Set 1	Motor activity (PND 13, 17, 22, 58)	10-12/sex	12/sex	11-12/sex	11-13/sex
	Detailed clinical/FOB (PND 1, 11, 21, 35, 45, 60)	12/sex	12/sex	11-12/sex	11-13/sex
Set 2	Auditory startle habituation (PND 23/24, 58)	10-11/sex	11-12/sex	11-12/sex	10-13/sex
Set 3	Auditory startle pre-pulse inhibition (PND 23/24, 58)	11/sex	11-13/sex	11-12/sex	11-13/sex
Sets 4 and	Learning and memory (PND 23/24, set 4; PND 58/59, set 5)	10-11/sex	12/sex	11-12/sex	11-13/sex

TABLE 1. Study design					
Experimental parameter		Dietary concentration (ppm)			
		0	250	700	2100
5					
Set 6	Gross necropsy and brain measurements (PND 21)	10-13/sex	10-13/sex	11/sex	12/sex

Data for offspring taken from text table p. 27, MRID 46062301.

5. Dose selection rationale: Dietary concentrations were chosen following consultation of scientists between the sponsor, US EPA, and the testing laboratory. In a range-finding study in rats (HLS report no. MTU 214/023366), groups of 8 mated females were administered 0, 700, 1400, 2800, or 4900 ppm in the diet from gestation day 6 through lactation day 20. Dietary concentrations of 2800 and 4900 ppm produced mild maternal toxicity, increased offspring mortality, and marked adverse clinical signs in the offspring. Further details of the effects were given in the protocol review (July31, 2002), but the MRID number for the range-finding study was not identified. A dose level of 2800 ppm was considered as possibly excessive based on decreased pup viability of 23% at that dose. No maternal or offspring effects were noted at 1400 ppm (pup viability was 98.4%). Therefore, dietary concentrations selected for the developmental neurotoxicity study were 0, 250, 700, and 2100 ppm (in accordance with the protocol review).

6. Dosage administration: Etofenprox was administered to female CD rats in the diet at levels of 0, 250, 700 or 2100 ppm from gestation day 6 through postnatal day 20. Direct dosing of the offspring was not considered necessary as it is known that the unmetabolized parent compound is present in rat milk (Protocol review, July 21, 2002). The data were not included in the current study report but were located in MRID 40449723. The following is an excerpt section from the MRID 40449723 revised DER executive summary (date: May 4, 2000) for etofenprox specifically concerning metabolism in pregnant rats and placental transfer:

> To measure quantitative transplacental transfer of radioactivity in pregnant rats, 10 pregnant rats were dosed once a day from gestation day 10 to gestation day 16 at 30 mg/kg/day. Two rats were sacrificed at 4, 24, 48, 72, and 120 hours after the final dose. Mammary tissues contained 87.4 µg/g at 4 hours post last dose and 32.4 µg/g at 120 hours post last dose. Adrenal glands concentrations were 61.5 µg/g at 4 hours post last dose and 5.74 µg/g at 120 hours post last dose. Liver concentrations were 27.2 µg/g at 4

hours post dose and 1.55 µg/g 120 hours post last dose. Placental concentrations were 4.7 µg/g at 4 hours post last dose and 0.17 µg/g at 120 hours post last dose. Mean fetal concentrations of two experiments were 1.65 µg/g at 4 hours and were 0.12 µg/g at 120 hours post last dose.

To measure qualitative transplacental transfer of radioactivity in pregnant rats, 5 pregnant rats were dosed once a day from gestation day 10 to gestation day 16 at 30 mg/kg/day. One rat was sacrificed at 4, 24, 48, 72, and 120 hours after the final dose. Sections of the animals were observed by autoradiography. Autoradiography of pregnant rats was similar to male rats dosed for 7 days. Maximum radioactivity was detected at 4 hours post last dose with highest concentration observed in the fat, GI tract, bile ducts, and mammary tissue.

To measure transfer of radioactivity into milk of nursing mothers, the stomach contents of suckling pups was measured. Three pregnant rats were dosed once daily for 14 days at 30 mg/kg/day beginning at gestation day 18. Dosing continued until approximately 9 days after parturition. Four additional undosed mothers were used to provide naive pups. Naive pups were allowed to suckle dosed mothers for one hour followed by pup sacrifice. Additional stomach milk samples were obtained at days 4, 6, 9, 10, 11, 12, 14, and 16 of lactation. At sacrifice, the stomach contents of the pup were removed and weighed. The concentration of radioactivity in stomach of pups ranged from 41.3 to 88.3 µg/g. Maternal plasma concentrations at same time were 1.9 to 3.6 µg/mL. This data indicates that bioconcentration of radioactivity occurred in milk.

7. **Dosage preparation and analysis:** Dietary formulations were prepared weekly. For the premix, the required amount of test article was weighed out and melted at 40°C then stirred into corn oil at a final concentration in the premix of 2% corn oil. The resultant premix was stirred into plain diet and further diet was added. The premix was then passed through a 1 mm sieve and/or ground in a coffee grinder/food processor (if necessary). The final premix was prepared by dilution with diet and blending in a Turbula mixer for approximately 100 revolutions. The required dietary concentrations were achieved by diluting the premix with diet and blending each mixture in a Turbula mixer for 100 revolutions. The final concentration of corn oil for the test diets was less than 0.175%. The control diet was similarly prepared to contain the same concentration of corn oil as the 2100 ppm diet. Concentrations of the test substance in the diet were measured by HPLC with UV detection during the first week of gestation and the first week of lactation. Homogeneity and stability of the test article the diet were determined in a previous study using concentrations of 100 and 5000 ppm (HLS report

no. MTU 222/023183); these data were not included in the current report and the concentrations are different than the ones used in this study.

Results:
Homogeneity analysis: Homogeneity was noted as confirmed in the previous study, however these data were not included in the report.

Stability analysis: The test article was shown stable in the diet for 15 days when stored at 21°C; data were not included.

Concentration analysis: Absence of test article was confirmed in the control diet. Mean concentrations of the test diets were ±6% of nominal.

Note, due to lack of data for homogeneity and stability analyses it is unknown if the test article in the diets was adequate. This is a **major deficiency**.

C. **OBSERVATIONS:**

1. **In-life observations:**

a. **Maternal animals:** All animals were checked twice daily for clinical signs, mortality, or moribundity. Each animal was subjected to a complete physical examination on gestation days 0, 7, 13, and 20 and on lactation days 1, 7, 14, and 21.

Ten dams per group were observed (by observers blind to the treatment group) outside the home cage during the gestation dosing period (days 12 and 18) and during the lactation dosing period (days 4, 11, and 21). The arena was cleaned after each animal was tested. The following functional observations were recorded.

	Functional observations–Maternal animals
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence or absence of piloerection and exophthalamus, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal

	movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

Individual maternal body weight was recorded on gestation days 0, 3, 6, 10, 14, 17, and 20 on lactation days 1, 4, 7, 11, 14, 17, and 21. Food consumption was measured on gestation days 0-2, 3-5, 6-9, 10-13, 14-16, and 17-19, and on lactation days 1-3, 4-6, 7-10, 11-13, 14-16, and 17-20. Food consumption measurements may have included consumption by the pups, especially during lactation week 3.

From gestation day 20, dams were checked three times daily for evidence of parturition. They were permitted to deliver and rear offspring until postnatal day 21. Numbers of live and dead offspring were recorded during parturition.

b. **Offspring**:

1) **Litter observations**: Offspring were examined approximately 24 hours after birth and the number of live and dead pups, individual body weight, sex ratio, and clinical signs were recorded. Litters were examined daily throughout lactation for clinical signs and mortality. In addition, offspring were observed daily through PND 28 for general clinical signs and mortality.

   On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible); excess pups were killed and discarded.

2) **Developmental landmarks**: Beginning on postnatal day 32, male offspring were examined daily for balanopreputial separation. Beginning on postnatal day 28, female offspring were examined daily for vaginal patency. The body weight at attainment was recorded.

3) **Detailed observations**: Offspring were examined for clinical signs twice daily. Beginning on PND 28, each animal was subjected to a full physical examination once a week. Individual offspring body weight data were recorded on PNDs 1, 4, 7, 11, 14, 17, 21, and 28 and once weekly thereafter. Food consumption by the offspring was not measured.

4) **Neurobehavioral evaluations**: Observations and the schedule for those observations are summarized as follows from the report. Note that equipment types and descriptions used for the behavioral tests were not provided in the study report. This is an **deficiency**.

i) **Functional observational battery (FOB)**: On postnatal days 4, 11, 21, 35, 45, and 60, a minimum of 10 offspring/sex/group was examined outside the home cage in an FOB assessment by observers blind to the treatment groups. On postnatal days 4 and 11, the animals were tested for surface righting reflex then observed in the open field for one minute. In the open field, PND 4 pups were assessed for activity, distance traveled, maximum pivoting angle, and any physical abnormalities in appearance or gait while PND 11 pups were assessed for activity, number of rearings, grooming, urination, and physical abnormalities. Methods on days 21, 35, 45, and 60 were similar to the procedures used for the dams. On PND 21, animals were tested before the dam was sent for necropsy.

	FUNCTIONAL OBSERVATIONS- Offspring
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence or absence of piloerection and exophthalamus, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

ii) **Motor activity testing**: Motor activity was evaluated on days 13, 17, 22, and 60 in the same animals used in the FOB. Animals were placed individually in plastic cages and were continuously monitored over a 1-hour period. An automated activity monitoring system collected data over successive 6-minute intervals by recording the frequency of infra-red light beam interruptions. Low beam detectors monitored ambulatory activity while high beam

detectors monitored rearing activity. Due to the small size of the animals on PNDs 13 and 17, a raised insert was placed in each activity cage on these days.

iii) **Auditory startle habituation and pre-pulse inhibition:** These tests were performed on a minimum of 10 offspring/sex/dose on PNDs 23/24 and 58, using an automated system. For both tests, the peak amplitude (g) was measured using a sensitive force platform and a measure of latency (msec) was used as a measure of the startle response.
AUDITORY STARTLE HABITUATION: Animals were acclimated for 5 minutes to background noise and were then presented with the startle stimulus at 12-second intervals during 5 consecutive blocks of 10 trials. The startle stimulus consisted of 40 millisecond bursts of white noise at approximately105 dB against a background noise level of 70 dB.

PRE-PULSE INHIBITION OF STARTLE: Amplitude and latency responses were recorded for 10 trials with a pre-pulse of sound immediately preceding the startle stimulus and for 10 trials without a pre-pulse. The 20 trials were presented in pseudo-random order with inter-trial intervals of 10, 12, 14, or 16 seconds. The startle stimulus consisted of a 50 millisecond burst of white noise at approximately 118 dB. The pre-pulse consisted of a 50 millisecond pulse of white noise at approximately 85 dB preceding the startle stimulus by 150 milliseconds.

iv. **Learning and memory testing:** Learning and memory testing was performed in a minimum of 10 offspring/sex/dose on PNDs 23/24 and 58 using a Morris water maze. The water-filled swimming maze consisted of a circular pool constructed of white plastic (90 cm diameter, 30 cm deep at days 23/24, and 140 cm diameter, 45 cm deep at days 58/59). The maze was filled with water approximately 29±3°C made opaque with a non-toxic opacifier. A platform 6 cm square for weanling rats and 10 cm square for adult rats was located at a fixed point in the pool, concealed approximately 1.5 cm below the surface of the water. Three starting points were identified at the perimeter of the pool. A number of visual cues were placed on the walls of the pool and a number of cues outside the pool were also available to assist learning. A series of 3 trials was conducted on each of 4 consecutive days. On the first trial the rat was placed on the escape platform for 30 seconds prior to

testing. The animal was then placed into the water at the perimeter of the pool and allowed a maximum of 90 seconds to swim to the platform. A different starting point was used for each trial. The time to reach the platform and the number of quadrants of the pool crossed were recorded. The rat was allowed to remain on the platform for 30 seconds after each trial. If the animal failed to find the platform within 90 seconds, it was placed on the platform for 30 seconds and a latency of 90 seconds recorded.

5) Opthalomology: Because eye abnormalities were observed grossly in pups, ophthalmoscopic examinations were conducted during post-weaning on selected offspring on October 18, 2002 to ensure that animal welfare was not adversely affected. Seven animals were examined and photographed: 4 males (1 mid-dose and 3 high-dose) and 3 females (all high-dose). No further details were given regarding the examination procedure.

2. Postmortem observations:

a. Maternal animals: Maternal animals were sacrificed by carbon dioxide inhalation on lactation day 21 and subjected to gross necropsy. The brain was removed and weighed, and together with any abnormal tissues was retained in fixative.

b. Offspring: Offspring were sacrificed on PNDs 21 or 65±2. Those animals not allocated for neuropathological examination were killed by carbon dioxide inhalation, examined grossly, and the brain removed, weighed and fixed in 10% neutral buffered formalin. The offspring selected for brain weight or neuropathological evaluation were subjected to postmortem examinations as described below.

At postnatal day 21, 10-13 pups/sex/group were sacrificed by intraperitoneal injection of a lethal dose of barbiturate and perfused via the left ventricle with fixative (gluteraldehyde and paraformaldehyde). Following perfusion, the animals were examined grossly. The brain was collected, weighed, and preserved in fixative. Brain length was measured between the rostral part of the cerebral hemispheres and the most caudal part of the cerebellum and width was measured at the widest part of the cerebral hemispheres. Brains from all dose groups were embedded in paraffin and were sectioned for control and high-dose animals. Tissues were sectioned at 4-5 μm and stained with hematoxylin and eosin. Five coronal sections and

one mid-sagittal section from control and high-dose animals were examined microscopically.

The following brain morphometric measurements were performed:

Neocortex thickness (distance from the pial surface to the top of the white matter along a line perpendicular to a tangent of the pial surface at the point where the cortex exhibits its greatest thickness)

Hippocampus thickness (greatest dorsal-ventral thickness)

Corpus callosum (thickness at the midline)

Cerebellum (width of the pyramis folia perpendicular to its long axis at the midpoint between its tip and base)

On postnatal day 65±2, 10 rats/sex/dose were were sacrificed by intraperitoneal injection of a lethal dose of barbiturate and perfused via the left ventricle with fixative (gluteraldehyde and paraformaldehyde). Following perfusion, the animals were examined grossly. The brain was collected, weighed, and preserved in fixative. Brain length was measured between the rostral part of the cerebral hemispheres and the most caudal part of the cerebellum and width was measured at the widest part of the cerebral hemispheres. The brain, spinal cord, both eyes with optic nerves, peripheral nerves (tibial and sciatic), dorsal root fibers and ganglia, ventral root fibers, and gastrocnemius muscle were collected and preserved in fixative.

The following central and peripheral nervous system tissues were dissected and preserved in paraffin (CNS tissues) or plastic (PNS tissues): five coronal sections and one mid-sagittal section of the brain, cervical and lumbar sections of the spinal cord, eyes, optic nerves, gastrocnemius muscle, dorsal root ganglia and fibers, and sciatic and tibial nerves. Tissues from all dose groups were embedded; however, only control and high-dose tissues were examined unless effects warranted examination of low- and mid-dose samples. Paraffin-embedded tissues were sectioned at 4-5 μm and stained with hematoxylin and eosin. Plastic-embedded tissues were sectioned at 2 μm and stained with toluidine blue.

Detailed morphometric evaluation of the neocortex, corpus callosum, hippocampus, and folia of cerebellum was conducted as follows:

Neocortex thickness (distance from the pial surface to the top of the white matter along a line perpendicular to a tangent of the pial surface at the point where the cortex exhibits its greatest thickness)

Hippocampus thickness (greatest dorsal-ventral thickness)

Corpus callosum (thickness at the midline)

Cerebellum (width of the pyramis folia perpendicular to its long axis at the midpoint between its tip and base)

D. **DATA ANALYSIS:**

1. **Statistical analyses**: Frequency data were analyzed using a Mantel test for a trend in proportions and a pairwise Fisher's Exact test for each dose group against the control if 75% of the data were the same value. Continuous data were initially analyzed for homogeneity of variance using Bartlett's test. If Bartlett's was not significant, parametric analysis (not stated, assumed to be ANOVA) was applied followed by Williams' test or Dunnett's test. If Bartlett's was significant, logarithmic and square-root transformations were tried followed by Shirley's test. For offspring brain weight data, homogeneity of variance was assessed using Bartlett's test followed by Behrens-Fisher test or Dunnett's test for pairwise comparisons. Brain morphometry data were analyzed using the Student's t test.

   The reviewer does not think that the statistical analyses were entirely appropriate. For continuous data (e.g., body weight) after variance homogeneity had been assessed, the data should have been analyzed by Analysis of Variance before pairwise comparisons were made.

2. **Indices**:

   a. **Reproductive indices**: The following reproductive index was calculated from breeding and parturition records of animals in the study:
   Gestation index = (Number of live litters/Number pregnant) × 100

b. **Offspring viability indices**: The following viability (survival) indices were calculated from lactation records of litters in the study:

Post-implant. survival index = (Number of offspring/Number of implant. sites) × 100

Live birth index = (Number of live offspring/Total number of offspring born) × 100

Viability index = (Number of live offspring at PND 4/Number of live offspring at PND 1) × 100

Lactation index = (Number of live offspring on day of examination/Number of live offspring on PND 4 after culling) × 100

3. Positive and historical control data: Only positive control FOB data in pre-weaning pups was submitted. Actual positive and historical control data for other endpoints are not presented and no references to such data were given in the report. It is assumed that these data sets have been submitted to EPA. (Please Note: The acute oral neurotoxicity, MRID 45932301, positive control data for observational methods and histopathological changes were provided on paraoxon, MRID 44163001, and acrylamide, MRID 44163002, by Hazleton Wisconsin Inc., performed in 1996. However, as of 11-09-2004 other data sets have not been identified.)

   A positive control FOB study (MRID 46213901, February 24, 2004, Huntingdon Life Sciences Ltd, England, Study Director D.P. Myers, rat species Crl:CD® (SD)BR IGS) in pre-weaning rats was submitted to show that the arenas used and parameters evaluated were appropriate for PND 4 and 11 pups. Groups of 10 pups/sex/group were administered vehicle control (0.9% saline), 3 mg/kg/day of Di-isofluoro-phosphate, or 2 mg/kg/day of D-amphetamine by gavage on PNDs 4 and 11. Additional groups of 10/sex served as untreated controls. FOB testing was performed approximately 30 minutes after dosing on each day. On both testing days, each animal was evaluated for surface righting reflex and scored as 1 (immediate, up to 2 sec), 2, (slow, 3-5 sec), or 3 (fail, more than 5 sec). The animal was then placed in an open arena and observed for one minute. On PND 4 the 18-cm diameter arena was subdivided into concentric circles and number of sections entered, maximum distance traveled, and maximum pivoting angle were recorded. On PND 11 the 30×21-cm arena was divided into nine equal section and number of sections entered, number of rearings, frequency of grooming (scored 0, 1, or 2), and occurrence of urination were recorded. In addition, on both days, physical condition, locomotor coordination, and any other abnormal behaviors were recorded.

Di-isofluoro-phosphate: Two males were found dead on PND 5. Body weight and body weight gain was not affected by treatment. On PND 4 mean surface righting reflex score was slightly lower in both sexes with statistical significance ($p \leq 0.05$) attained for females (males: 2.2 vs 2.5; females: 2.2 vs 2.8). Results for maximum pivoting angle on PND 4 were highly variable with large standard deviations about the mean. The maximum distance traveled and the number of sections entered was similar to the control on PND 4. On PND 11 no effects were observed for surface righting reflex, grooming (none observed in any group), activity, or rearing (observed in one control female).

D-amphetamine: All animals survived to scheduled sacrifice. Body weight gain was transiently decreased during PNDs 4-7 compared to vehicle controls (males: 4.8 g vs 5.3 g for controls; females: 4.4 g vs 4.8 g for controls). Surface righting reflex was similar to controls on both days. Results for maximum pivoting angle on PND 4 were highly variable with large standard deviations about the mean. On PND 4 females had increases for maximum distance traveled (1.4 cm vs 0.0 cm for controls) and activity (1.3 sections vs 0.4 sections for the controls). Activity was increased on PND 11 in both males and females with statistical significance ($p \leq 0.05$) attained for males (males: 13.4 sections vs 8.9 sections for controls; females: 15.1 sections vs 10.7 sections for controls). Grooming was not observed in any animal. On PND 11 rearing was observed in two treated males and three treated females compared with one vehicle control female.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations**: No dams were found dead or were sacrificed in moribund condition during gestation or lactation. Mean rearing count for high-dose animals was consistently greater than that of controls at each testing day (3.5-6.0 vs 1.7-4.0 for controls). Hair loss and brown staining were common findings on animals in the treated and control groups. No other treatment-related clinical signs of toxicity were observed during gestation or lactation at either cage-side observation or during the FOB.

Open Field: Mean rearing counts for Fo females orally administered etofenprox via the diet.

Test day	Dietary concentration (ppm)			
	0	250	700	2100
DM 12	2.5	1.5	3.7	3.5 (40%)
DM 18	1.7	2.3	2.1	3.9* (129%)
DL 4	3.2	3.2	3.1	4.1 (28%)
DL 11	2.4	3.8	2.8	5.2 (117%)
DL 21	4.0	3.4	3.3	6.0 (50%)

Data obtained from text p. 75 - 81, MRID 46062301. DM= day after mating, DL= day of lactation. n = 9-10.
Significantly different from control: *= $p<0.05$

2. **Body weight and food consumption**: Selected group mean body weight and food consumption values for pregnant or nursing dams are summarized in Table 2. No treatment-related effects on body weight or food consumption were observed during gestation or lactation. Weight gain by the mid- and high-dose groups was significant less (14%) than that of the control group during gestation on DG 6-10 and greater (21-61%) than that of the controls during lactation for the high-dose group.

TABLE 2. Selected mean (±SD) maternal body weight and food consumption				
Observation/study interval	0 ppm	250 ppm	700 ppm	2100 ppm
Gestation (n= 24)				
Body wt. GD 0 (g)	276 ± 30	284 ± 27	276 ± 23	270 ± 23
Body wt. GD 6 (g)	308 ± 31	320 ± 27	308 ± 26	303 ± 25
Body wt. GD 14 (g)	353 ± 33	363 ± 29	350 ± 25	345 ± 24
Body wt. GD 20 (g)	430 ± 40	443 ± 34	430 ± 30	423 ± 29
Wt. gain GDs 6-10	21 ± 6	20 ± 4	18* ± 3	18* ± 5 (↓14%)
Wt. gain GDs 6-20 (g)	123 ± 17	124 ± 13	121 ± 13	121 ± 15
Food consumption GDs 6-9 (g/rat/day)	27 ± 3	29 ± 2	27 ± 2	26 ± 2
Food consumption GDs 17-19 (g/rat/day)	31 ± 3	33 ± 3	31 ± 3	31 ± 3
Lactation (n=23-24)				
Body wt. lactation day 1 (g)	335 ± 30	337 ± 27	330 ± 26	320 ± 23
Body wt. lactation day 4 (g)	348 ± 33	354 ± 28	344 ± 27	340 ± 23

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TABLE 2. Selected mean (±SD) maternal body weight and food consumption				
Observation/study interval	**0 ppm**	**250 ppm**	**700 ppm**	**2100 ppm**
Body wt. lactation day 14 (g)	368 ± 33	380 ± 29	364 ± 28	365 ± 27
Body wt. lactation day 21(g)	356 ± 34	367 ± 26	354 ± 29	356 ± 23
Wt. gain LDs 1-4 (g)	15 ± 8	16 ± 8	15 ± 8	**21* ± 8** (↑40%)
Wt. gain LDs 1-7 (g)	19 ± 6	20 ± 8	17 ± 8	23 ± 10 (↑21%)
Wt. gain LDs 1-11 (g)	28 ± 12	36 ± 11	32 ± 10	**37* ± 11** (↑32%)
Wt. gain LDs 1-14 (g)	35 ± 16	43 ± 12	36 ± 16	**45* ± 14** (↑28%)
Wt. gain LDs 1-17 (g)	33 ± 16	43 ± 12	36 ± 16	**44* ± 12** (↑33%)
Wt. gain LDs 1-21 (g)	23 ± 15	29 ± 14	26 ± 13	**37** ± 11** (↑61%)
Food consumption lactation days 1-3 (g/day)	37 ± 5	37 ± 4	37 ± 7	37 ± 4
Food consumption lactation days 17-20 (g/day)	71 ± 12	74 ± 9	73 ± 9	73 ± 9

Data obtained from Tables 12-14, pp. 83-85, MRID 46062301.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

3. **Test substance intake**: Based on maternal food consumption and body weight and nominal dietary concentrations, the doses expressed as mean daily mg test substance/kg body weight during the gestation and lactation periods are presented in Table 3. Maternal doses were not calculated for lactation days 14-20 due to feed intake by the offspring.

TABLE 3. Mean maternal test substance intake (mg/kg body weight/day)			
Period	**250 ppm**	**700 ppm**	**2100 ppm**
Gestation			
Gestation days 6-9	22	60	175
Gestation days 10-13	21	58	171
Gestation days 14-16	20	57	167
Gestation days 17-19	20	54	164
Overall average dose	21	57	169
Lactation			
Lactation days 1-3	27	77	238
Lactation days 4-6	31	88	262

Lactation days 7-10	41	112	340
Lactation days 11-13	46	130	393
Overall average dose	28.4	79.2	238

Data obtained from text p. 41 and Table 15, p. 86, MRID 46062301.

4. **Reproductive performance**: Reproductive performance is summarized in Table 4. Gestation length, number of live litters born, and gestation index were similar between the treated and control groups.

TABLE 4. Reproductive Performance

Observation	0 ppm	250 ppm	700 ppm	2100 ppm
Number assigned (pregnant)	24	24	24	24
Gestation length (days)				
22	15	14	16	18
22.5	5	4	4	5
23	4	6	4	1
Implantations/dam	16.0 ± 2.0	16.1 ± 2.8	15.7 ± 2.3	16.6 ± 2.0
Number of live litters born	24	24	24	24
Gestation index (%)	100	100	100	100

Data obtained from Tables 16 and 18, pp. 87 and 89, respectively, MRID 46062301.

5. **Maternal postmortem results**: No treatment-related abnormalities were found at maternal necropsy. Brain weight was similar between the treated and control groups.

B. **OFFSPRING**:

1. **Viability and clinical signs**: Litter size and viability (survival) results of pups during lactation are summarized in Table 5. No treatment-related effect on the number of litters, live litter size, sex ratio, or live birth, viability, or lactation indices was observed. The number of stillborn pups was not given. One control dam and one mid-dose dam had total litter loss on days 6 and 1, respectively. These litters were not used in the calculations for litter size or survival but should have been included; this omission does not compromise the integrity of the study.

TABLE 5. Litter size and viability

Observation	0 ppm	250 ppm	700 ppm	2100 ppm
Number of live litters	24	24	24	24
Total litter loss	1	0	1	0
Mean no. of viable pups[a]				
Day 1	15.2 ± 2.0	15.5 ± 2.4	14.8 ± 2.3	15.3 ± 2.2
Day 4 (precull)	14.2 ± 2.2	14.6 ± 2.8	14.0 ± 2.6	15.2 ± 2.2
Day 4 (postcull)	8.0 ± 0.0	7.9 ± 0.3	8.0 ± 0.0	8.0 ± 0.0
Day 21	7.4 ± 1.6	7.8 ± 0.7	7.9 ± 0.5	7.5 ± 1.0
Post-implantation survival index (%)[a]	93.9	95.7	92.2	92.4
Live birth index (%)[a]	96.6	98.4	99.2	99.2
Viability index (%)[a]	97.1	95.6	95.9	99.8
Lactation index (%)[a]	92.9	97.9	98.4	93.8
Sex ratio day 1 (%male)	49.7	52.1	45.3	48.2

Data obtained from Tables 18-20, pp. 89-91, respectively, MRID 46062301.
[a]Group mean does not include dams with total litter loss.

Treatment-related clinical signs of toxicity in the offspring included eye lesions and body injuries. Combinations of one or both eyes large/prominent, dark, and opaque were observed in five pups from three mid-dose litters and in twelve pups from nine high-dose litters. This lesion was also observed in one control pup and two low-dose pups. Eye abnormalities were first observed in both low-dose pups and most of the mid-dose pups post-weaning, whereas all but one of the high-dose pups displayed the lesion during lactation. Five of these high-dose pups were killed between days 18-28 and one mid-dose pup was killed on day 31 for reasons of animal welfare. In addition, injuries or trauma such as cuts, bleeding, swelling, redness, or bruises were seen on a total of 11 pups from high-dose litters and on 5 pups from mid-dose litters compared with only 1-2 findings in the low-dose and control groups.

Principal offspring clinical signs- group incidences in the F1 generation exposed to Etofenprox.

	Number of offspring (litters) affected in group			
Dietary concentration	0 ppm	250 ppm	700 ppm	2100 ppm
Clinical signs				
Initial eye abnormality				
One or both eyes large/prominent and dark	-	-	1(1)	8(6)
One or both eyes large/prominent	-	1(1) PW	1(1) PW	1(1)
One eye large and opaque	1(1)	1(1) PW	-	-
One or both eyes opaque	-	-	3(1) PW	1(1)
One or both eyes dark	-	-	-	2(2), 1(1) PW
Cut/bleeding on tail	-	-	-	2(2)
Reddened/swollen/bruised area(s) on tail	1(1)	-	3(1)	4(3)
Cut/bleeding on toes/paws	-	-	1(1)	2(2)
Swollen/bruised/reddened paw(s)	1(1)	1(1)	2(2)	5(4)

Data from Table 21, page 92, MRID 46062301. PW - Eye changes first recorded post weaning.

To ensure that animal welfare was not adversely affected, further ophthalmoscopic examinations were conducted during post-weaning on selected offspring on October 18, 2002 (page 1289). Seven animals were examined and photographed (photographs were not submitted): 4 males (1 mid-dose and 3 high-dose) and 3 females (all high-dose). Findings were dark blood filling the anterior chamber, evidence of sclera haemorrhage and one lens haemorrhage, opaque lens, cataract just evident in two and total cataract in one animal.

2. **Body weight**: Selected offspring body weight data are given in Table 6 for the lactation interval and Table 7 for the post-weaning interval. Body weight and body weight gain for male and female offspring were similar between the treated and control groups throughout the study.

TABLE 6. Mean (±SD) pre-weaning pup body weight and body weight gain (g)				
Day of age/ interval	0 ppm	250 ppm	700 ppm	2100 ppm
Males				
0	6.5 ± 0.6	6.6 ± 0.8	6.5 ± 0.7	6.3 ± 0.7
4 (post-cull)	8.9 ± 1.1	9.0 ± 1.3	9.0 ± 1.3	8.6 ± 1.1

7	14.8 ± 2.3	14.9 ± 1.8	14.4 ± 2.5	13.8 ± 2.0
14	32.2 ± 3.3	32.5 ± 2.9	31.3 ± 3.7	30.7 ± 3.4
21	51.0 ± 5.4	51.4 ± 5.8	48.9 ± 6.3	48.6 ± 5.7
Wt. gain days 1-4	2.4 ± 0.6	2.5 ± 0.8	2.5 ± 0.9	2.4 ± 0.7
Wt. gain days 1-21	44.5 ± 5.1	44.8 ± 5.3	42.5 ± 6.0	42.4 ± 5.3
N	23	24	23	24
Females				
0	6.1 ± 0.6	6.1 ± 0.8	6.1 ± 0.7	6.0 ± 0.6
4 (post-cull)	8.4 ± 1.0	8.6 ± 1.1	8.4 ± 1.2	8.3 ± 1.0
7	13.8 ± 2.1	14.1 ± 1.8	13.8 ± 2.1	13.2 ± 2.1
14	30.8 ± 3.8	31.5 ± 2.9	30.2 ± 3.6	29.3 ± 3.7
21	49.0 ± 5.0	49.2 ± 5.6	46.8 ± 6.1	46.8 ± 5.7
Wt. gain days 1-4	2.3 ± 0.6	2.4 ± 0.6	2.3 ± 0.7	2.3 ± 0.7
Wt. gain days 1-21	42.9 ± 4.7	43.1 ± 5.1	40.7 ± 5.6	40.8 ± 5.3
N	23	24	23	24

Data obtained from Tables 22-25, pp. 93-96, respectivly, MRID 46062301.

TABLE 7. Mean (±SD) post-weaning offspring body weight and body weight gain (g)				
Day of age	**0 ppm**	**250 ppm**	**700 ppm**	**2100 ppm**
Males				
28	86 ± 9	88 ± 8	84 ± 10	83 ± 9
35	134 ± 15	141 ± 13	133 ± 14	133 ± 12
49	251 ± 25	265 ± 22	249 ± 23	253 ± 20
63	358 ± 33	374 ± 32	359 ± 32	363 ± 28
Wt. gain days 28-63	272 ± 26	**286*** ± 27	275 ± 25	280 ± 24
N	23	24	23	24
Females				
28	79 ± 8	80 ± 9	77 ± 10	76 ± 9
35	119 ± 13	122 ± 12	117 ± 14	116 ± 12
49	181 ± 19	186 ± 15	180 ± 19	181 ± 16
63	227 ± 24	233 ± 20	226 ± 26	229 ± 20
Wt. gain days 28-63	149 ± 19	152 ± 17	150 ± 20	153 ± 18
N	23	24	23	24

Data obtained from Tables 49 and 50, pp. 129 and 130, respectively, MRID 46062301.
Significantly different from control: *$p \leq 0.05$.

3. **Developmental landmarks:**

a. **Sexual maturation**: Age at preputial separation of males and vaginal opening of females was not affected by treatment (Table 8). Mean body weight at attainment was similar between the treated and control groups for males and females.

TABLE 8. Mean (±SD) age of sexual maturation (days)

Parameter	0 ppm	250 ppm	700 ppm	2100 ppm
N (M/F)	76/73	80/81	74/82	78/76
Preputial separation (males)	46.3 ± 2.6	45.0 ± 2.3	46.1 ± 3.0	46.0 ± 2.6
Body Weight at Attainment	120.1	119.0	116.2	113.6
Vaginal opening (females)	35.3 ± 2.5	34.6 ± 2.3	35.0 ± 2.3	34.6 ± 1.8
Body Weight at Attainment	227.6	228.9	225.2	225.2

Data obtained from Tables 51 and 52, pp. 131 and 132, respectively, MRID 46062301.

b. **Developmental landmarks:** Due to an eye abnormality the pupil closure reflex could not be assessed in the right eye of two high-dose males on PND 35. For all other animals tested, the pupil reflex was not affected by treatment. Other developmental landmarks, such as incisor eruption, pinna unfolding, and fur growth, were not monitored.

4. **Behavioral assessments:**

a. **Functional observational battery**: Data are presented in Table 9. No treatment-related effects were found during in-hand or arena observations of offspring on any test day (PND 4, 11, 21, 35, 45, or 60). During in-hand observations, some high-dose animals were noted to have eye abnormalities similar to those described during general clinical examinations. The mean activity counts were lower for mid- and high-dose treated males, but only statistically significant for PND 45. The lower mean activity counts seen on PND 45 in males were determined to be incidental and not toxicologically significant due to the lack of dose response, absence of such decreases on the other days at any dose in this sex or in females at any dose on any test day.

TABLE 9. FOB- Arena Observations, Mean (±S.D.) activity count				
Test day	0 ppm	250 ppm	700 ppm	2100 ppm
Males				
PND 35	10.1 ± 5.1	10.9 ± 5.1 (↑8%)	6.7 ± 4.8 (↓34%)	8.2 ± 5.5 (↓19%)
PND 45	9.2 ± 4.1	6.6 ± 3.7 (↓28%)	**3.9* ± 3.3** (↓58%)	**5.8* ± 4.7** (↓37%)
PND 60	6.5 ± 4.0	7.2 ± 3.6 (↑11%)	3.2 ± 1.8 (↓51%)	5.1 ± 3.5 (↓22%)
Females				
PND 35	11.5 ± 5.4	8.7 ± 3.8 (↓24%)	10.3 ± 5.6 (↓10%)	8.6 ± 3.3 (↓25%)
PND 45	10.1 ± 4.8	10.2 ± 5.5 (↑1%)	6.2 ± 3.7 (↓39%)	10.6 ± 5.3 (↑5%)
PND 60	13.5 ± 3.6	9.9 ± 3.5 (↓27%)	9.9 ± 5.0 (↓27%)	12.8 ± 4.6 (↓5%)

Data obtained from Tables 29-31, pp. 101-103, MRID 46062301. S.D. = standard deviation. Calculated by the reviewer.]
n = 10-13/sex/dose.

b. **Motor activity**: Mean total activity counts for motor from high beam and low beam activities are given in Tables 10 and 11, respectively. For motor activity from high beam breaks, habituation was apparent in some groups on all four test days, including PND 13 when activity levels were relatively low, and in all groups by PND 17. For activity from low beam breaks, habituation was apparent on all test days. No treatment-related effects on overall or interval motor activity were noted in females on any testing day. On PND 58 high-dose males had lower activity levels (25%) than controls with statistical significance attained for low beam activity. Both activity counts for the high-dose males were consistently lower than those of controls for all intervals on PND 58.

TABLE 10. Mean (±S.D.) motor activity data on high beam (total rearing activity counts for session)				
Test day	0 ppm	250 ppm	700 ppm	2100 ppm
Males				
PND 13	0.8 ± 1.0	0.3 ± 1.2	1.4 ± 2.8	0.2 ± 0.6 (↓75%)
PND 17	21.3 ± 24.3	71.9 ± 61.3	39.1 ± 54.7	30.9 ± 43.9 (↑45%)
PND 22	53.5 ± 58.4	27.8 ± 18.0	19.4 ± 10.4	36.2 ± 50.8 (↓32%)
PND 58	281.0 ± 124.7	298.0 ± 81.0 (↑6%)	270.5 ± 77.7 (↓4%)	218.4 ± 117.8 (↓22%)
Females				

TABLE 10. Mean (±S.D.) motor activity data on high beam (total rearing activity counts for session)				
Test day	0 ppm	250 ppm	700 ppm	2100 ppm
PND 13	1.2 ± 2.9	0.6 ± 1.2	1.0 ± 1.5	1.1 ± 2.3 (↓8%)
PND 17	29.5 ± 26.6	49.1 ± 87.5	44.4 ± 67.8	41.4 ± 51.0 (↑40%)
PND 22	34.6 ± 23.0	43.1 ± 20.2	45.7 ± 37.4	31.7 ± 35.1 (↓8%)
PND 58	282.4 ± 107.5	275.8 ± 105.4 (↓2%)	224.3 ± 74.4 (↓21%)	262.6 ± 43.9 (↓7%)

Data obtained from Tables 35-38, pp. 107-116, MRID 46062301. n = 10-13/sex/dose.

TABLE 11. Mean (±S.D.) motor activity data on low beam (total cage floor activity counts for session)				
Day of age	0 ppm	250 ppm	700 ppm	2100 ppm
Males				
PND 13	375.9 ± 294.3	261.0 ± 270.1	266.3 ± 162.3	435.2 ± 372.0 (↑16%)
PND 17	235.2 ± 117.6	513.5 ± 332.7	340.7 ± 370.0	517.9 ± 403.5 (↑120%)
PND 22	248.9 ± 250.0	169.2 ± 80.3	129.0 ± 62.5	171.0 ± 143.4 (↓31%)
PND 58	1094.4 ± 371.8	1082.5 ± 290.1 (↓2%)	1034.4 ± 243.0 (↓6%)	**822.6* ± 336.1** (↓25%)
Females				
PND 13	249.7 ± 171.6	225.5 ± 99.1	202.9 ± 170.9	315.6 ± 353.6 (↑26%)
PND 17	474.6 ± 223.9	407.2 ± 454.2	384.7 ± 397.6	531.7 ± 353.5 (↑12%)
PND 22	180.2 ± 89.5	229.6 ± 130.5	240.1 ± 178.0	195.6 ± 167.3 (↑9%)
PND 58	1207.3 ± 346.3	1534.9 ± 468.9 (↑27%)	1098.4 ± 242.9 (↓9%)	1360.6 ± 240.3 (↑13%)

Data obtained from Tables 35-38, pp. 107-116, MRID 46062301. n = 10-13/sex/dose.
Significantly different from control: *p ≤ 0.05.

c. Auditory startle reflex:

AUDITORY STARTLE HABITUATION: Peak amplitude data are summarized in Table 12 and latency data are summarized in Table 13. High-dose males and females had greater peak amplitudes than those of controls for all trial blocks on both testing days, with the exception of males on PND

23/24 during trials 1-10. Statistical significance was attained for several blocks of trials especially for females on PND 58. Correspondingly, the latency to peak response was lower for high-dose males and females compared with the controls, but only on PND 58.

PRE-PULSE INHIBITION OF STARTLE: Auditory startle pre-pulse inhibition data are given in Table 14. All treated and control groups showed an inhibition of the peak amplitude of response when presented with a pre-pulse before the startle stimulus; in contrast the latency was not affected by a pre-pulse. On PND 58 mid- and high-dose males and females showed a greater inhibition of response and males a longer latency than that of the control group.

TABLE 12. Auditory startle reflex peak amplitude data (mean g ±S.D.)

Test day	Trial number	0 ppm	250 ppm	700 ppm	2100 ppm
			Males		
PND 23/24	1-10	198.3 ± 55.0	206.2 ± 84.3	170.7 ± 65.5	192.5 ± 115.8
	11-20	163.7 ± 43.9	160.8 ± 66.5	175.5 ± 65.9	186.1 ± 114.7
	21-30	142.3 ± 50.3	164.2 ± 56.9	168.3 ± 66.3	177.0 ± 131.4
	31-40	152.2 ± 37.2	164.1 ± 72.9	159.5 ± 64.9	168.0 ± 131.9
	41-50	147.9 ± 49.5	150.8 ± 67.3	142.6 ± 73.2	153.3 ± 112.7
PND 58	1-10	383.4 ± 149.9	325.7 ± 190.3	403.6 ± 155.1	492.4 ± 296.4
	11-20	317.9 ± 94.2	307.6 ± 144.2	330.6 ± 123.0	413.6 ± 229.9
	21-30	311.1 ± 137.1	282.9 ± 123.8	316.6 ± 104.2	440.5 ± 238.0
	31-40	294.8 ± 145.8	316.1 ± 137.7	311.0 ± 128.8	385.4 ± 202.0
	41-50	292.0 ± 161.6	338.2 ± 148.1	310.6 ± 137.4	**471.2* ± 270.1**(↑61%)
			Females		
PND 23/24	1-10	164.2 ± 56.0	149.5 ± 62.1	166.8 ± 68.9	186.5 ± 60.1
	11-20	148.9 ± 58.8	126.5 ± 47.3	136.8 ± 66.1	181.6 ± 76.3
	21-30	131.0 ± 56.7	123.4 ± 47.7	142.9 ± 62.3	174.0 ± 78.6
	31-40	121.9 ± 34.1	120.1 ± 44.5	141.0 ± 48.4	**168.9* ± 75.3** (↑38%)
	41-50	128.7 ± 52.9	121.5 ± 43.3	141.4 ± 55.6	179.6 ± 83.6
PND 58	1-10	310.9 ± 102.9	335.5 ± 164.4	332.9 ± 117.0	408.4 ± 157.2

TABLE 12. Auditory startle reflex peak amplitude data (mean g ±S.D.)

Test day	Trial number	0 ppm	250 ppm	700 ppm	2100 ppm
	11-20	235.3 ± 80.0	259.5 ± 116.1	280.6 ± 80.8	**381.8** ** ± 145.8 (↑62%)
	21-30	239.3 ± 94.2	299.7 ± 102.7	280.0 ± 92.5	**342.8** * ± 130.9 (↑43%)
	31-40	241.4 ± 63.8	273.9 ± 131.5	298.4 ± 93.4	**352.5** * ± 150.6 (↑46%)
	41-50	257.8 ± 71.5	246.0 ± 97.8	304.9 ± 89.7	**372.4** ** ± 126.8 (↑44%)

Data obtained from Tables 41 and 43, pp. 121 and 123, respectively, MRID 46062301. n = 10-13/sex/dose
Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

TABLE 13. Auditory startle reflex latency data (mean msec ±S.D.)

Test day	Trial number	0 ppm	250 ppm	700 ppm	2100 ppm
		Males			
PND 23/24	1-10	19.1 ± 6.1	24.2 ± 5.9	23.0 ± 6.3	18.8 ± 7.2
	11-20	19.4 ± 5.3	25.3 ± 4.4	231 ± 6.2	21.1 ± 7.4
	21-30	23.0 ± 4.3	26.4 ± 5.3	23.4 ± 4.3	21.0 ± 4.4
	31-40	21.7 ± 5.3	24.7 ± 5.7	24.7 ± 2.7	23.1 ± 6.7
	41-50	23.0 ± 8.4	26.6 ± 6.4	24.2 ± 4.2	20.8 ± 8.5
PND 58	1-10	21.1 ± 8.0	20.8 ± 7.6(↓1%)	**14.8** * ± 5.5 (↓31%)	**13.9** * ± 5.7(↓34%)
	11-20	16.4 ± 5.2	17.9 ± 8.3	13.6 ± 5.3	15.8 ± 6.2
	21-30	20.2 ± 7.2	13.5 ± 5.9	14.3 ± 6.0	15.5 ± 8.6
	31-40	18.4 ± 5.2	15.3 ± 5.5 (↓17%)	**13.6** * ± 3.6 (↓26%)	**12.0** * ± 6.6(↓35%)
	41-50	17.6 ± 7.4	13.3 ± 4.4	15.5 ± 4.6	13.2 ± 4.0
		Females			
PND 23/24	1-10	22.2 ± 5.0	20.5 ± 8.1	22.6 ± 4.9	22.1 ± 4.7
	11-20	22.2 ± 7.8	22.2 ± 9.2	21.4 ± 7.3	22.0 ± 4.4
	21-30	20.6 ± 8.1	19.6 ± 5.3	22.2 ± 4.5	22.4 ± 5.0
	31-40	21.5 ± 5.3	21.7 ± 8.1	22.6 ± 5.7	23.0 ± 5.5
	41-50	23.4 ± 8.9	21.7 ± 6.1	23.6 ± 4.8	22.6 ± 4.7
PND 58	1-10	18.1 ± 4.8	16.0 ± 5.3	16.4 ± 4.6	15.1 ± 4.6

TABLE 13. Auditory startle reflex latency data (mean msec ±S.D.)

Test day	Trial number	0 ppm	250 ppm	700 ppm	2100 ppm
	11-20	16.7 ± 6.0	15.5 ± 6.9	16.0 ± 6.6	15.2 ± 4.2
	21-30	17.1 ± 4.0	14.2 ± 6.8	15.6 ± 5.2	15.1 ± 3.4
	31-40	15.6 ± 6.2	15.7 ± 7.0	16.5 ± 4.5	13.3 ± 3.2
	41-50	16.5 ± 5.5	16.7 ± 9.9	16.9 ± 4.7	14.4 ± 4.7

Data obtained from Tables 42 and 44, pp. 122 and 124, respectively, MRID 46062301. n = 10-13/sex/dose
Significantly different from control: *p ≤ 0.05.

TABLE 14. Auditory startle reflex pre-pulse inhibition data (mean ±S.D.)

Endpoint	Pre-pulse	0 ppm	250 ppm	700 ppm	2100 ppm
		Males PND 23/24			
Amplitude (g)	no	234.0 ± 69.4	237.2 ± 98.5	227.6 ± 75.7	252.3 ± 107.2
	yes	181.4 ± 66.7	196.4 ± 87.6	196.6 ± 77.5	199.0 ± 82.1
	% inhibition	22.4 ± 18.3	17.2 ± 10.5	14.0 ± 17.4	21.1 ± 10.1
Latency (ms)	no	16.3 ± 5.8	20.3 ± 4.4	19.5 ± 7.6	16.4 ± 7.5
	yes	17.4 ± 7.9	21.2 ± 4.7	17.4 ± 8.3	17.6 ± 7.5
		Females PND 23/24			
Amplitude (g)	no	241.0 ± 59.7	253.8 ± 94.9	232.4 ± 80.6	225.7 ± 105.8
	yes	198.4 ± 50.6	198.0 ± 82.6	175.3 ± 61.5	188.3 ± 81.9
	% inhibition	17.6 ± 10.2	22.7 ± 12.7	23.5 ± 13.7	15.0 ± 14.9
Latency (ms)	no	19.4 ± 9.1	16.3 ± 8.3	20.0 ± 6.2	16.5 ± 5.7
	yes	19.1 ± 8.1	16.5 ± 6.9	20.4 ± 6.1	18.0 ± 5.4
		Males PND 58			
Amplitude (g)	no	575.7 ± 175.0	586.9 ± 307.3	612.6 ± 153.4	632.7 ± 231.3
	yes	447.0 ± 144.9	462.1 ± 248.0	451.0 ± 187.4	416.0 ± 227.4
	% inhibition	18.1 ± 29.8	17.2 ± 21.1 (↓6%)	27.8 ± 20.4 (↑54%)	36.6 ± 25.7(↑102%)
Latency (ms)	no	14.7 ± 7.0	16.1 ± 7.2	14.7 ± 5.6	15.7 ± 5.7
	yes	12.4 ± 6.7	14.6 ± 3.4	13.5 ± 4.7	**17.4* ± 6.4** (↑40%)
		Females PND 58			
Amplitu	no	450.9 ± 171.4	426.2 ± 135.7	526.4 ± 153.4	**599.1* ±**

TABLE 14. Auditory startle reflex pre-pulse inhibition data (mean ±S.D.)

Endpoint	Pre-pulse	0 ppm	250 ppm	700 ppm	2100 ppm
de (g)					139.6(↑33%)
	yes	362.4 ± 124.7	306.6 ± 98.4	375.8 ± 164.8	419.0 ± 132.7
	% inhibition	16.7 ± 21.8	25.5 ± 20.0 (↑51%)	30.3 ± 15.7 (↑81%)	29.6 ± 16.7 (↑77%)
Latency (ms)	no	15.3 ± 6.8	16.5 ± 6.8	15.1 ± 6.3	13.3 ± 7.3
	yes	14.1 ± 4.2	16.7 ± 6.5	15.4 ± 6.9	13.9 ± 8.5

Data obtained from Tables 45, 46, 47, and 48, pp. 125-128, respectively, MRID 46062301. n = 10-13/sex/dose
Significantly different from control: *$p \leq 0.05$.
"no" refers to stimulus without pre-pulse, "yes" refers to stimulus with pre-pluse.

d. Learning and memory testing: Performance results in the Morris water maze are given in Tables 15 and 16 for males and females, respectively. No treatment-related effects on learning and memory were observed. All groups demonstrated learning of the position of the escape platform as evidenced by decreases in trial time, the number of failed trials, and the number of sectors entered over the four days of testing.

TABLE 15. Morris water maze performance - males (mean ± S.D.)

Test day/parameter		0 ppm	250 ppm	700 ppm	2100 ppm
		PND 23/24			
Day 1	Trial time (sec)	69.9 ± 17.9	71.5 ± 13.0	71.9 ± 15.5	77.4 ± 16.1
	No. failed trials	1.5 ± 1.1	2.0 ± 0.7	2.0 ± 0.9	2.3 ± 0.8
	No. sector entries	16.5 ± 2.7	18.9 ± 3.4	17.5 ± 2.9	17.9 ± 2.8
Day 2	Trial time (sec)	56.9 ± 21.9	56.9 ± 13.9	57.4 ± 20.2	65.3 ± 14.5
	No. failed trials	1.1 ± 0.9	1.0 ± 0.6	1.3 ± 1.0	1.4 ± 1.1
	No. sector entries	14.9 ± 4.1	17.6 ± 4.4	15.9 ± 7.1	18.5 ± 3.6
Day 3	Trial time (sec)	38.2 ± 23.2	41.2 ± 18.8	49.8 ± 25.2	50.6 ± 27.8
	No. failed trials	0.6 ± 1.0	0.4 ± 0.7	1.1 ± 1.0	1.1 ± 1.2
	No. sector entries	10.7 ± 5.3	13.2 ± 4.4	15.8 ± 6.4	15.5 ± 8.0
Day 4	Trial time (sec)	41.6 ± 25.3	33.0 ± 20.3	40.0 ± 25.9	36.2 ± 18.7
	No. failed trials	0.7 ± 0.9	0.4 ± 0.8	0.6 ± 1.0	0.5 ± 0.7
	No. sector entries	11.2 ± 4.6	11.0 ± 4.4	12.5 ± 6.5	11.6 ± 4.4
		PND 58			
Day 1	Trial time (sec)	65.1 ± 12.0	69.5 ± 15.4	74.3 ± 15.3	65.2 ± 14.9
	No. failed trials	1.2 ± 0.9	1.3 ± 0.8	2.0 ± 0.9	1.2 ± 0.7
	No. sector entries	12.9 ± 2.7	14.1 ± 2.9	14.4 ± 3.0	13.3 ± 3.4
Day 2	Trial time (sec)	31.8 ± 19.6	37.8 ± 24.0	49.6 ± 21.7	37.3 ± 18.3
	No. failed trials	0.5 ± 0.7	0.5 ± 1.0	0.9 ± 1.0	0.5 ± 0.9
	No. sector entries	7.9 ± 3.4	9.4 ± 3.8	10.9 ± 3.3	9.3 ± 3.0
Day 3	Trial time (sec)	20.0 ± 15.3	29.3 ± 21.3	32.3 ± 25.0	22.2 ± 19.6
	No. failed trials	0.0 ± 0.0	0.3 ± 0.7	0.4 ± 0.9	0.2 ± 0.6
	No. sector entries	6.5 ± 5.1	7.3 ± 4.0	7.4 ± 4.4	6.1 ± 3.7
Day 4	Trial time (sec)	14.7 ± 7.0	22.3 ± 22.7	18.4 ± 14.2	15.6 ± 7.4
	No. failed trials	0.0 ± 0.0	0.2 ± 0.4	0.2 ± 0.4	0.0 ± 0.0
	No. sector entries	4.6 ± 1.4	5.8 ± 4.0	5.4 ± 3.7	5.0 ± 1.9

Data obtained from Tables 39 and 40, pp. 117 and 119, respectively, MRID 46062301.
N = 10-13/sex/dose

TABLE 16. Morris water maze performance - females (mean ± S.D.)

Test day/parameter		0 ppm	250 ppm	700 ppm	2100 ppm
		PND 23/24			
Day 1	Trial time (sec)	83.5 ± 9.4	72.5 ± 17.3	79.8 ± 13.2	75.5 ± 18.1
	No. failed trials	2.5 ± 0.5	1.6 ± 0.9	2.3 ± 0.8	2.0 ± 0.9
	No. sector entries	21.4 ± 3.9	19.4 ± 4.9	19.9 ± 4.2	18.3 ± 5.4
Day 2	Trial time (sec)	64.9 ± 21.6	52.1 ± 22.0	66.3 ± 20.5	58.3 ± 27.3
	No. failed trials	1.5 ± 1.2	1.0 ± 1.0	1.5 ± 1.2	1.2 ± 1.1
	No. sector entries	18.4 ± 4.1	15.4 ± 6.4	18.6 ± 5.2	15.1 ± 5.9
Day 3	Trial time (sec)	39.9 ± 23.1	37.5 ± 12.8	42.9 ± 19.1	45.1 ± 31.3
	No. failed trials	0.5 ± 1.0	0.3 ± 0.5	0.5 ± 1.0	1.0 ± 1.3
	No. sector entries	12.0 ± 5.3	13.4 ± 5.7	14.0 ± 5.7	13.8 ± 7.5
Day 4	Trial time (sec)	32.2 ± 20.9	25.5 ± 21.4	45.3 ± 31.7	26.5 ± 15.4
	No. failed trials	0.4 ± 0.8	0.3 ± 0.9	0.8 ± 1.3	0.2 ± 0.4
	No. sector entries	11.3 ± 6.3	9.1 ± 6.3	15.4 ± 10.4	9.3 ± 4.1
		PND 58			
Day 1	Trial time (sec)	69.9 ± 14.2	65.6 ± 22.8	79.5 ± 10.6	70.1 ± 7.9
	No. failed trials	1.9 ± 0.7	1.8 ± 1.1	2.3 ± 0.8	1.6 ± 0.5
	No. sector entries	15.9 ± 3.2	15.2 ± 5.1	17.5 ± 2.9	15.4 ± 3.4
Day 2	Trial time (sec)	40.6 ± 15.3	58.0 ± 21.3	48.4 ± 21.8	43.4 ± 21.0
	No. failed trials	0.8 ± 0.6	1.3 ± 1.0	0.8 ± 1.0	0.7 ± 0.6
	No. sector entries	10.9 ± 4.7	14.1 ± 5.0	11.7 ± 3.7	9.8 ± 4.5
Day 3	Trial time (sec)	28.9 ± 21.4	48.1 ± 18.5	31.6 ± 22.9	35.7 ± 24.1
	No. failed trials	0.3 ± 0.7	0.8 ± 0.8	0.3 ± 0.9	0.6 ± 0.9
	No. sector entries	7.2 ± 4.2	11.0 ± 4.6	7.7 ± 3.7	7.5 ± 4.3
Day 4	Trial time (sec)	26.8 ± 19.2	24.6 ± 12.9	23.6 ± 8.4	26.4 ± 17.7
	No. failed trials	0.4 ± 0.5	0.2 ± 0.4	0.1 ± 0.3	0.3 ± 0.6
	No. sector entries	6.6 ± 3.7	6.5 ± 3.1	6.8 ± 2.7	7.1 ± 4.0

Data obtained from Tables 39 and 40, pp. 118 and 120, respectively, MRID 46062301.
N = 10-13/sex/dose

e. <u>Ophthalmology</u>: Complete ophthalmoscopic examinations were not done on all animals. However, in order to assess the welfare of some of the offspring showing eye abnormalities on clinical examination, three males and three females from the high-dose group and one male from the mid-dose group were examined further. Unilateral intraoccular hemorrhage was observed in all of these animals, but they were judged able to continue on study.

5. **Postmortem results**:

a. **Brain weight**: Mean brain weight data are presented in Table 17. No treatment-related effects on absolute brain weights were observed at weaning or study termination.

TABLE 17. Mean (±SD) brain weight data in offspring

Parameter	0 ppm	250 ppm	700 ppm	2100 ppm
Males				
PND 21				
Terminal body weight (g)	52.2 ± 7.0	49.4 ± 6.5	47.6 ± 5.3	49.7 ± 6.3
Brain weight (g)	1.48 ± 0.07	1.43 ± 0.11	1.49 ± 0.08	1.48 ± 0.07
PND 63-67				
Terminal body weight (g)	367.5 ± 31.1	386.2 ± 35.1	378.1 ± 27.6	372.8 ± 23.5
Brain weight (g)	2.02 ± 0.20	2.03 ± 0.11	2.02 ± 0.10	2.01 ± 0.09
Females				
PND 21				
Terminal body weight (g)	49.6 ± 2.9	49.0 ± 5.2	45.9 ± 7.3	45.9 ± 5.3
Brain weight (g)	1.44 ± 0.08	1.46 ± 0.09	1.47 ± 0.08	1.41 ± 0.07
PND 63-67				
Terminal body weight (g)	246.2 ± 29.5	237.9 ± 17.2	228.7 ± 26.2	227.0 ± 20.8
Brain weight (g)	1.90 ± 0.06	1.85 ± 0.10	1.82 ± 0.21	1.87 ± 0.07

Data obtained from Tables 53A, 53B, 57A, and 57B, pp. 133, 134, 139, and 140, respectively, MRID 46062301.
n = 10-13/sex/dose
Significantly different from control: *p ≤ 0.05.

b. **Cholinesterase activity**: Cholinesterase activity was not measured in this study.

C. **Neuropathology:**

1. **Macroscopic examination**: Eye abnormalities were confirmed at necropsy of offspring on PNDs 21 and 63-67. No other treatment-related effects were reported for male or female offspring at PND 21. At study termination, the incidence of renal pelvic dilation was 17-24 males in each treated group compared with 7 control males. Predominantly, only one kidney was affected. When bilateral pelvic dilation was considered, an increased incidence was observed for males in the high-dose group (7 vs 0-1 in the control, low-, and mid-dose groups). Females were not similarly affected.

2. **Microscopic examination**: No significant treatment-related effects were noted on PND 21; minimal degeneration in the granular layer of the cerebellum was observed in two high-dose animals. At study termination, no treatment-related effects were observed in the tissues of the central or peripheral nervous system; minimal degenerative changes were observed at similar incidences in the control and high-dose groups. Two high-dose males with an opaque eye were found to have lenticular degeneration with associated retinal folds and minimal accumulations of macrophages in the iris or uvea.

Eye Histopathology Findings

Finding Description	Control	250 ppm	700 ppm	2100 ppm
number examined	10	10	10	10
retinal rosettes/folds	2	0	0	3
lenticular degeneration	0	0	0	2
pigmented macrophages - iris	0	0	0	2
pigmented macrophages - uvea	0	0	0	1

Data obtained from Tables 60, p. 143, MRID 46062301.

3. **Brain Morphometry:** Data for males and females are summarized in Tables 18 and 19, respectively. No treatment-related differences in any measurement were observed for either sex at PNDs 21 or 65. On PND 65, hippocampal thickness was significantly greater for high-dose males and significantly less for high-dose females compared to their respective control groups. For males, all high-dose individual values were within the range of the control values. For females, the control group value was greater than that measured in two preceding studies (1.80 and 1.97 mm) conducted by the testing facility. .

TABLE 18. Mean (±SD) morphometric data in male offspring

Parameter	0 ppm	250 ppm	700 ppm	2100 ppm
Day 21				
Brain length (mm)	18.2 ± 0.7	17.5 ± 0.5	17.8 ± 0.4	18.1 ± 0.5
Brain width (mm)	14.5 ± 0.4	14.6 ± 0.5	14.8 ± 0.4	14.4 ± 0.4
Neocortex (mm)	1.73 ± 0.10	--	--	1.67 ± 0.09
Hippocampus (mm)	1.65 ± 0.14	--	--	1.58 ± 0.16
Corpus callosum (mm)	0.19 ± 0.04	--	--	0.21 ± 0.06
Cerebellum (mm)	0.80 ± 0.07	--	--	0.83 ± 0.07
PND 65				
Brain length (mm)	21.1 ± 0.3	21.1 ± 0.5	20.9 ± 0.5	21.2 ± 0.4

TABLE 18. Mean (±SD) morphometric data in male offspring				
Parameter	**0 ppm**	**250 ppm**	**700 ppm**	**2100 ppm**
Brain width (mm)	15.4 ± 0.5	15.5 ± 0.5	15.4 ± 0.5	15.6 ± 0.3
Neocortex (mm)	1.80 ± 0.11	--	--	1.88 ± 0.12
Hippocampus (mm)	1.88 ± 0.21	--	--	**2.05* ± 0.12** (↑9%)
Corpus callosum (mm)	0.32 ± 0.09	--	--	0.30 ± 0.06
Cerebellum (mm)	0.81 ± 0.06	--	--	0.80 ± 0.06

Data obtained from Tables 54, 55, 58, and 59, pp. 135, 136, 141, and 142, respectively, MRID 46062301.
N = 10/sex/dose
Significantly different from control: *$p \leq 0.05$.

TABLE 19. Mean (±SD) morphometric data in female offspring				
Parameter	**0 ppm**	**250 ppm**	**700 ppm**	**2100 ppm**
Day 21				
Brain length (mm)	17.9 ± 0.4	17.7 ± 0.5	17.8 ± 0.5	17.8 ± 0.5
Brain width (mm)	14.5 ± 0.7	14.3 ± 0.3	14.4 ± 0.3	14.4 ± 0.3
Neocortex (mm)	1.66 ± 0.11.	--	--	1.64 ± 0.15
Hippocampus (mm)	1.62 ± 0.11	--	--	1.59 ± 0.14
Corpus callosum (mm)	0.19 ± 0.04	--	--	0.22 ± 0.07
Cerebellum (mm)	0.79 ± 0.07	--	--	0.85 ± 0.14
PND 65				
Brain length (mm)	20.4 ± 0.5	20.2 ± 0.6	20.6 ± 0.7	20.5 ± 0.4
Brain width (mm)	15.0 ± 0.4	15.1 ± 0.3	15.2 ± 0.2	15.1 ± 0.3
Neocortex (mm)	1.91 ± 0.13	--	--	1.90 ± 0.08
Hippocampus (mm)	1.99 ± 0.13	--	--	**1.81** ± 0.10** (↓9%)
Corpus callosum (mm)	0.27 ± 0.07	--	--	0.23 ± 0.04
Cerebellum (mm)	0.83 ± 0.10	--	--	0.78 ± 0.07

Data obtained from Tables 54, 55, 58, and 59, pp. 135, 136, 141, and 142, respectively, MRID 46062301.
N = 10/sex/dose
Significantly different from control: **$p \leq 0.01$.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS**: The study author concluded that maternal toxicity was evident at 700 ppm as reduced weight gain during gestation, and at 2100 ppm as reduced weight gain during gestation and increased rearing activity. Offspring toxicity was evident as ocular abnormalities at 700 and 2100 ppm and as slightly decreased survival during the final week of lactation and injuries at 2100 ppm. The LOAEL for functional developmental of offspring was 2100 ppm based on higher mean startle response amplitudes in females and differences in motor activity and latency to peak startle response in males; the NOAEL was 700 ppm. The NOAEL for histomorphological development of central and peripheral nerve tissues was at least 2100 ppm.

B. **REVIEWER COMMENTS**: In dams, no treatment-related effects on mortality, clinical signs, body weight, food consumption, or necropsy findings were noted. The reviewer disagrees with the study author that the lower body weight gain by the high-dose dams during gestation (only on GD 6-10) was treatment-related. The mean difference in weight gain between the high-dose group and the control group was a total of 3 g which is neither biologically nor toxicologically significant. Statistical significance was attained but the reviewer does not think that the analysis was appropriate. However, the high-dose related greater body weight gain during the entire lactation period was notable. The slightly greater rearing count by the high-dose females is of questionable toxicological significance if viewed as a single study effect; however effects on rearing were also observed in the rat subchronic neurotoxicity study and rat developmental study.

Therefore, the maternal NOAEL is 700 ppm (57 mg/kg/day). The maternal LOAEL is 2100 ppm (169 mg/kg/day, HDT) based on greater body weight gains during the lactation period and increased incidence of rearing behavior in the FOB.

Treatment had no adverse effects on survival, body weight, body weight gain, developmental landmarks, learning and memory, brain weights, brain morphology, or neuropathology. Treatment-related clinical signs of toxicity in the offspring included eye lesions and body injuries. Combinations of one or both eyes large/prominent, dark, and opaque were observed in one control pup, two pups from two low-dose litters, five pups from three mid-dose litters and in twelve pups from nine high-dose litters. Five of these high-dose pups were killed between days 18-28 and one mid-dose pup was killed on day 31 for reasons of animal welfare. Ophthalmoscopic examination of some of the affected pups revealed unilateral intraoccular hemorrhage. In addition, injuries or trauma such

as cuts, bleeding, swelling, redness, or bruises were seen on a total of 11 pups from high-dose litters and on 5 pups from mid-dose litters compared with only 1-2 findings in the low-dose and control groups.

No treatment-related effects on overall or interval motor or locomotor activity were seen in female offspring at any dose level on any test day or in male offspring at the low and mid dose groups at any test day. In the high dose males, however, there was a statistically significant lower motor activity level than controls on PND 58.

During auditory startle habituation testing, high-dose males and females had greater peak amplitudes than controls for nearly all trial blocks on both testing days, and latency to peak response was lower for high-dose males and females compared with the controls on PND 58. For pre-pulse inhibition on PND 58, high-dose males showed a greater inhibition of response and a longer latency than the control group.

The offspring systemic and neurotoxicity LOAEL is 700 ppm (57 mg/kg/day) based on eye abnormalities in both sexes. The offspring NOAEL is 250 ppm (21 mg/kg/day).

C. **STUDY DEFICIENCIES:**

- Homogeneity and stability analysis were not included in the report.

- Equipment types and descriptions used in the motor activity and the auditory startle tests were not provided in the study report.

- In the memory assessment, there was no evidence for recall/memory. Consequently, additional data are needed to confirm whether different animals were used at different time point and whether intra- or extra maze cues were used in the assessments.

This study is classified **Unacceptable/Non-Guideline** developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft). This study could be considered acceptable if the analytical data for homogeneity and stability analysis are provided. With the analytical data this study may be used for regulatory purposes. This study is considered non-guideline at this time due to deficiency in the memory assessment in the offspring and pending a comprehensive review of all available positive control data.

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